THE ROLE OF EXTENDED Vs CONVENTIONAL ECHOCARDIOGRAPHIC PARAMETERS TO QUANTIFY SEVERITY OF AORTIC STENOSIS

THE ROLE OF EXTENDED Vs CONVENTIONAL ECHOCARDIOGRAPHIC PARAMETERS TO QUANTIFY SEVERITY OF AORTIC STENOSIS

A Dissertation submitted in partial fulfilment of

D.M (Cardiology) Examination of the Tamil Nadu

Dr. M.G.R. UNIVERSITY, Chennai

To be held in August 2015

CONTENTS

Certificate

This is to certify that the dissertation entitled "**The role of extended Vs conventional echocardiographic parameters to quantify severity of aortic stenosis**" is the bona fide work of Dr. P. Sirish Chandra Srinath, towards the DM (Cardiology) degree Examination of the Tamil Nadu Dr.M.G.R University, Chennai to be conducted in August 2015.

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Certificate

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Declaration

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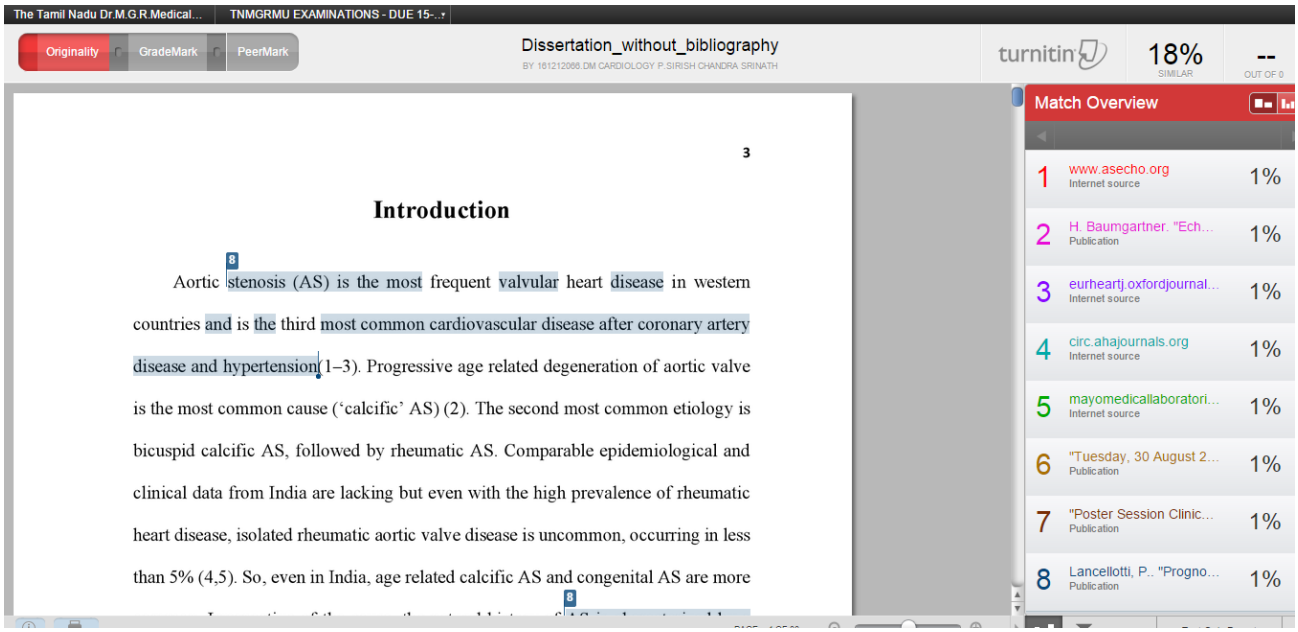
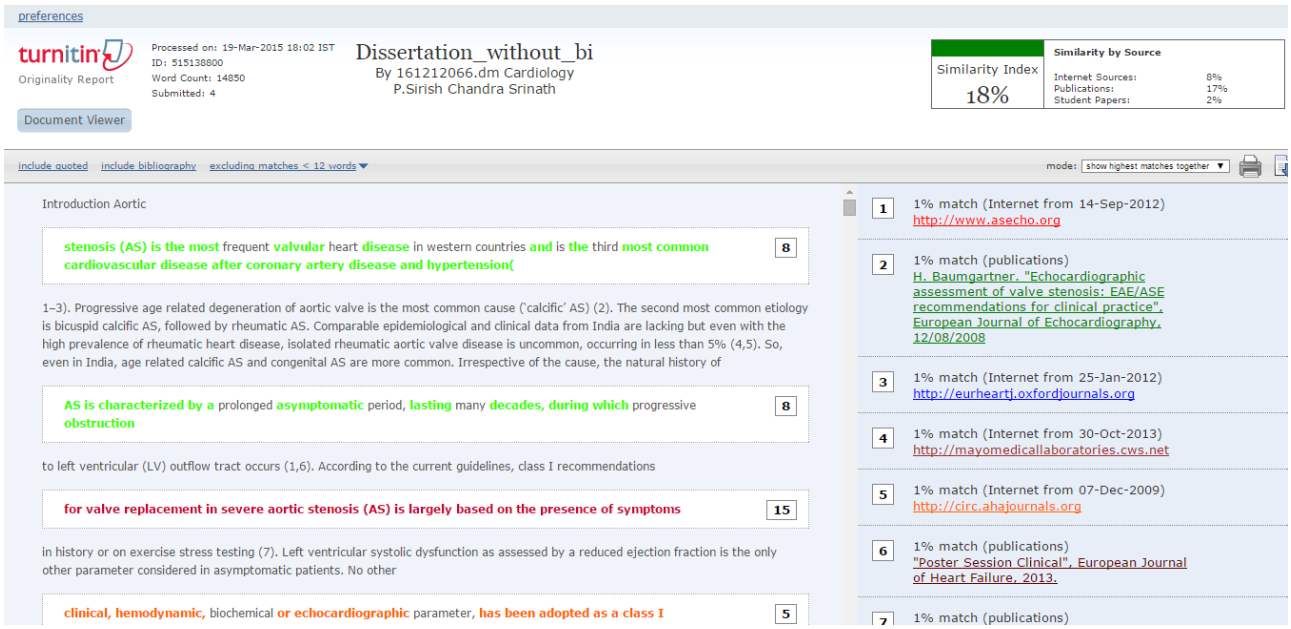
Originality certificate





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ABSTRACT

Aims: The objective of the study was to evaluate left ventricular (LV) strain by speckle tracking imaging and plasma NT-ProBNP in patients with moderate to severe aortic valve stenosis (AS).

Methods: Thirty-three patients with isolated AS with preserved ejection fraction (EF) and ten controls underwent assessment of symptoms, transthoracic echocardiography and measurement of plasma levels of NT-ProBNP.LV Strain and plasma NT-ProBNP were analysed to find differences and correlation with conventional echocardiographic parameters and clinical variables. These parameters were also studied for their strength to predict symptomatic status in these patients.

Results: Global longitudinal (GLS), global area (GAS) and global radial (GRS) strains were lower in patients with aortic stenosis (n=33; Median -13.0,-26.0 and 40.0 ,respectively) compared to controls (n=10; Median -20.4 ,-31.5 and 49.5,respectively;p <0.001 ,0.02 and 0.01 respectively).GLS,GAS and GRS were also lower in severe AS patients (n=27 ;Median -12.6,-25.0 and – 38.0,respectively) compared to moderate AS patients (n=6;Medain -19.8,-32.5 and 52.5 respectively; p=0.02,0.01 and0.03 respectively).GLS,GAS and GCS were lower in symptomatic (n=21;Median -11.6,-25.0 and 38.0) compared to asymptomatic (n=12;Median -16.45,-29.5 and 47.0 respectively; p=0.001,0.005 and 0.018 respectively) patients. Global circumferential strain (GCS) did not differ significantly between controls and AS patients or between subgroups of AS. There was a regional difference in strain with longitudinal strain in basal segments being decreased with preserved apical segmental longitudinal strain. Plasma NT-ProBNP

was higher in AS patients (Median 628.00 pg/ml) compared to controls (80.82 pg/ml; p<0.001). NT-ProBNP was higher in severe AS (Median 614.0 pg/ml) patients compared to moderate AS patients (Median 118.9 pg/ml) and symptomatic (Median 1191.0 pg/ml) compared to asymptomatic (Median 118.9 pg/ml) patients. Absolute value of GLS correlated strongly with LV mass index (r= -0.70; p<0.001) and NT-ProBNP correlated strongly with LA volume index (r= 0.74; p<0.001). Log-transformed NT-ProBNP correlated well with GLS (r= -0.63; p<0.001).Of all the variables NT-ProBNP was the best predictor of symptomatic status ; cut-off of 190.95 pg/ml has sensitivity of 90.5% and specificity of 91.7%.NT-ProBNP cut-off for predicting severe AS was 141.50 pg/ml with a sensitivity of 88.9% and specificity of 83.3%.

Conclusions: LV strain, especially GLS and plasma NT-ProBNP are affected early in patients with AS before the onset of symptoms and deterioration of LV function. Measurement of these variables to assess aortic stenosis patients may complement clinical and echocardiographic evaluation of these patients.

Key words: Aortic stenosis, Strain , NT-ProBNP

Introduction

Aortic stenosis (AS) is the most frequent valvular heart disease in western countries and is the third most common cardiovascular disease after coronary artery disease and hypertension(1–3). Progressive age related degeneration of aortic valve is the most common cause (‘calcific’ AS) (2). The second most common etiology is bicuspid calcific AS, followed by rheumatic AS. Comparable epidemiological and clinical data from India are lacking but even with the high prevalence of rheumatic heart disease, isolated rheumatic aortic valve disease is uncommon, occurring in less than 5% (4,5). So, even in India, age related calcific AS and congenital AS are more common. Irrespective of the cause, the natural history of AS is characterized by a prolonged asymptomatic period, lasting many decades, during which progressive obstruction to left ventricular (LV) outflow tract occurs (1,6). According to the current guidelines, class I recommendations for valve replacement in severe aortic stenosis (AS) is largely based on the presence of symptoms in history or on exercise stress testing (7). Left ventricular systolic dysfunction as assessed by a reduced ejection fraction is the only other parameter considered in asymptomatic patients. No other clinical, hemodynamic, biochemical or echocardiographic parameter, has been adopted as a class I recommendation for valve replacement in the absence of symptoms (or if not undergoing other cardiac surgery) in patients with isolated aortic stenosis.

In asymptomatic AS increased afterload results in progressive left ventricular hypertrophy and fibrosis, diastolic dysfunction and ultimately, intrinsic

myocardial dysfunction that becomes irreversible with duration (8–10). During the asymptomatic period, the risk of sudden death is similar to that of age matched general population (11). Hence, prophylactic surgery is not recommended and current guidelines recommend follow-up for these patients (7). There are several problems in this approach. First, the truly asymptomatic status is often difficult to assess, particularly in the elderly, who are not active (12). Patients may experience subtle symptoms and then unconsciously adjust activities to a level that does not produce symptoms and then deny symptoms (12). Exercise testing is recommended in such cases of severe AS with no or equivocal symptoms to decide on surgery, but is rarely performed in this setting, even in developed countries, as demonstrated by a survey of clinical practice in Europe (13). The slight but definite risk involved in exercise stress testing and difficulty of performing it in the elderly are important deterrents. Second, patients under follow-up may not immediately present when symptoms develop. Once symptomatic, these patients are at significant risk of rapid deterioration and sudden death while awaiting surgery and operative risk increases with symptom severity. Fifth, there are no good predictors of rapid progression of severity to symptomatic status (12). Myocardial structural changes and dysfunction continue to occur without symptoms and intrinsic myocardial dysfunction cannot be detected early if only ejection fraction is used as an index of myocardial performance. To complicate the issues, follow up in India is not systematic, putting these patients at risk of adverse events.

On the other hand, the operative risk, particularly in elderly patients, and prosthetic valve–related long-term morbidity and mortality prevents operating on all

asymptomatic patients with severe AS (14,15). Ideally, the decision for surgery should be late enough to outweigh the surgical risk and sufficiently early to avoid irreversible damage of the LV myocardium (14). The inter-patient variability in the progression of disease, the factors responsible for it and complexity of LV response to chronic obstruction are not completely understood (11). So a decision to operate in asymptomatic patients based on objective data is difficult to make. Therefore, newer parameters are needed to better predict the outcome in patients with AS. Risk stratification based on these parameters could thus help identify asymptomatic patients who would likely benefit from early elective surgery and at the same time avoid unnecessary interventions.

Recent advances in echocardiographic technology and identification of cardiac biomarkers have provided us with newer tools. Speckle tracking imaging and natriuretic peptides (which respond to wall stress) are the ones most commonly studied in the recent years in the setting of aortic stenosis. In the first place, this study helps in creating a cohort of patients with isolated AS in our setting and describe the clinical, echocardiographic and biochemical profile of these patients with particular reference to speckle tracking echocardiography and natriuretic peptides. Long-term follow up of these patients will give greater insight into the outcome, predictors of outcome and the role of above said parameters in this group of patients.

Aims and objectives

1) To evaluate left ventricular strain by speckle tracking imaging (STI) and plasma NT-ProBNP with in patients with moderate to severe aortic stenosis with preserved LV function.

2) To find correlation and differences between these newer parameters and conventional echocardiographic parameters.

3) To find the value of these parameters to predict symptomatic status in these patients.

Review of literature

Aortic stenosis: Etiology, pathophysiology and natural history

There are three main causes of valvular aortic stenosis (2). The most common cause being calcific aortic valve disease (age related calcific AS) which has a prevalence of 2% in adults more than 65 years of age. It shares risk factors with atherosclerotic arterial disease and has similar, but not same, pathophysiological basis (2,16). Multiple genetic polymorphisms have been linked to calcific AS, including vitamin D receptor, Apo lipoproteins and lipoprotein (a), similar to atherosclerotic vascular disease (17–21). The next most common cause is congenital bicuspid aortic valve (BAV) with superimposed calcification. BAV has 2% incidence in general population and most, if not all, patients develop at least some degree of aortic valve dysfunction during their lifetime. Most patients present with aortic stenosis beyond 50 years of age when superimposed calcification causes significant stenosis. In a large series of AS patients, BAV accounted for more than half of the total cases and two-thirds of patients aged less than 70 years (22). Even among patients over 70 years of age, BAV accounted for 40% of the cases. On an average patients with BAV require valve surgery ten years younger than age-related calcific AS and is also associated with 'aortopathy' which can present as coarctation or aneurysm.

Rheumatic heart disease involves aortic valve usually in conjunction with mitral valve involvement. Isolated aortic valve involvement is uncommon (< 5%)

(4,5). When AV alone is involved, it usually is a combination of regurgitation and stenosis with isolated rheumatic aortic stenosis (without significant mitral valve involvement or aortic incompetence) being a rare entity.

Congenital valvular aortic stenosis can be unicuspid, bicuspid or tricuspid. Most patients with unicuspid valve present in childhood (1). Rare individuals with tricuspid congenital AS can present late. Other rare causes of AS are alkaptonuria and homozygous type II hyperlipoproteinemia (familial hypercholesterolemia) presenting in childhood as a part of severe diffuse atherosclerosis (2).

Irrespective of the cause, the common pathophysiological characteristic is progressive obstruction to left ventricular ejection. The initial response to this increased afterload is replication of sarcomeres in parallel (8,23).This leads to increased wall thickness with normal or reduced LV diameter (concentric hypertrophy) and thereby reducing wall stress. Early in the disease process this hypertrophy is adaptive but there are limits to this adaptation. First, as the severity of obstruction increases a stage comes when even maximal hypertrophy cannot cope up with degree of obstruction and the LV function starts deteriorating ,called 'afterload mismatch' (8,24). In this situation the myocardial contractility is normal and if obstruction is relieved by surgery at the right time the ventricular function can be normalized. Second, the hypertrophy is inherently pathological with low capillary to myocyte ratio and reduced coronary flow reserve (25,26). There is also reduced perfusion pressure due to increased LVEDP. On the other hand the oxygen demand is increased due to increase in myocardial mass, systolic LV pressure and prolonged ejection. This mismatch causes myocardial ischemia especially in the subendocardial

region, even without obstructive coronary artery disease (25,27,28). Myocardial fibrosis starts in the subendocardium and progresses (9). In these patients, even in the presence of normal LV systolic function as indicated by preserved LV EF, the myocardial contractility is diminished and may not revert to normal even after surgery (27).

Natural history of AS fits into the schema of pathophysiological events just discussed. In their classic paper, Ross and Braunwald described the natural history of AS (6). There is a prolonged 'latent period' of few decades where progressive obstruction and adaptation go on together and patient is asymptomatic. Approximately 30% of these asymptomatic patients with severe AS will develop symptoms within 2 years of diagnosis (11). Among those patients who remain asymptomatic the risk of sudden cardiac death is less than 1% per year (11). Once LV dysfunction sets in, either due to afterload mismatch or depressed contractility due to fibrosis, the patient becomes symptomatic and once the symptoms appear there is rapid progression to death if untreated. The average time of death after onset of cardiac failure, syncope and angina is 2,3 and 5 years, respectively (6).

Echocardiographic assessment of aortic stenosis

Echocardiography has replaced cardiac catheterization as the standard for assessing aortic stenosis. The conventional echocardiographic parameters that have been well studied and validated, with wide acceptance and recommended for evaluation of AS severity by current guidelines are 1)Peak aortic jet velocity 2) Mean transaortic pressure gradient 3) Aortic valve area by continuity equation (7). Other

parameters have not been well validated and adopted widely, but the technology to assess these has been existent for many years. They are related to changes in LA and LV as a consequence of AS and include LV thickness, LV mass, LA size, LV systolic and diastolic dysfunction parameters. With the advent of new echocardiographic technologies like speckle tracking imaging (STI) and 3D echocardiography (3DE) multiple new parameters like myocardial strain and torsion dynamics are being studied .

Peak aortic jet velocity

The forward systolic velocity across the aortic valve is measured using continuous wave doppler (CWD) and peak jet velocity is defined as the highest velocity signal obtained from any window (29). So, multiple windows should be examined in order to determine the highest velocity. Careful patient and transducer position are important as velocity measurement assumes that the flow direction and ultrasound beam are parallel. Deviation from these rules results in velocity underestimation, so it is important that intercept angle is within 15° of parallel and 'angle correction' as a substitute to this should not be used (29–31).

Mean transaortic pressure gradient

The difference in mean systolic pressure between the left ventricle (LV) and aorta, or mean transvalvular pressure gradient, is another standard measure of stenosis severity (30,31). Gradients are calculated from velocity signal according to the modified Bernoulli's equation (as 4v2 where v is velocity) and the mean gradient is calculated by integrating the instantaneous gradients over the total ejection period.

There are multiple sources of error in measuring jet velocity and transaortic gradient (29). They include recording of and contamination by MR jet and improper alignment of the jet and ultrasound beam. Any error in velocity estimation results in an even greater error in gradients, as pressure is exponentially related to velocity by modified Bernoulli's equation (as 4v2). The pressure recovery (PR) phenomenon can also cause overestimation of pressure gradient, especially when aortic root is less than 3 cm (29).

Aortic valve area

Doppler velocity is flow dependent (29).So for any given orifice area, velocity (and the calculated gradient) increase with an increase in flow across the aortic valve. This can overestimate AV stenosis in high flow states like anemia, thyrotoxicosis or other hyperdynamic conditions. Underestimation of severity can occur in patients with low ejection fraction or low stroke volumes due to small chamber dimensions. Calculation of the aortic valve area (AVA) is helpful in these abnormal flow states (29). Aortic valve area is calculated based on the continuity equation whose principle is that volume flow (product of cross-sectional area and linear velocity, AV) remains constant throughout the flow. So when area increases velocity decreases and vice versa.

Calculation of AVA requires three measurements:

- Aortic valve velocity time integral (AV VTI)

● Left ventricular outflow tract (LVOT) diameter for calculation of its area, assuming circular geometry

● LVOT velocity time integral (LVOT VTI)

Accuracy in this method depends on the accurate measurement of the LVOT diameter, as area is related to the diameter to the power of two (1 mm error in LVOT diameter measurement results in an error of about 10 % in AVA) (29). The measurement variability for LVOT diameter ranges from 5% to 8% (29) .Another factor is the assumption of geometry of LVOT to be circular.

AS is a disease continuum, but is graded on the basis of above three parameters. Mild disease is characterised by a jet velocity less than 3 m/s, pressure gradient less than 25 mmHg, and AVA of 1.5 cm2 or more , and severe disease by a jet velocity more than 4 m/s, pressure gradient more than 40 mmHg, and AVA less than 1.0 cm2.1 Moderate AS falls between these values (7,32).

Other parameters

There are many surrogate markers of the effect of AS on left ventricle and left atrium. They have been studied in multiple smaller studies but are not widely adopted due to various reasons. They include LV thickness, LV mass, LA size, LV systolic and diastolic dysfunction parameters.

Left ventricular wall thickness and mass

As discussed earlier, LV hypertrophy is an adaptive mechanism in aortic stenosis. But the degree of LV hypertrophy is poorly correlated with the severity of

flow obstruction in multiple studies (33–35). This is because there are factors in addition to the pressure overload influencing the LV response. Age, gender and genetic variation in the renin–angiotensin system all play a probable role (33–36). Studies have also shown that 10–20% of patients with severe AS do not have LV hypertrophy (34,37).The prognostic value of LVH in patients with AS has been shown to be variable. Whereas multiple smaller studies showed poor prognosis with increasing LV mass, Otto et al. found that echocardiographic LV mass had no predictive value in asymptomatic patients (38–42). LV hypertrophy is defined as the LV mass index > 95 g/m2 for women and > 115 g/m2 for men as measured by echocardiography using Devereaux formula (43).

Global systolic function

The traditional parameters to assess LV systolic function include 2D ejection fraction(EF) and fractional shortening (FS).Both these are affected late in the disease course of AS either due to afterload mismatch or due to fibrosis. Patients with reduced EF overall have poorer prognosis even after surgery. The present study excludes patients with obvious LV systolic dysfunction as assessed by 2D EF or FS.

Diastolic function

Diastolic function can be assessed by a combination of doppler and tissue doppler techniques (44). The parameters studied are mitral diastolic flow velocities (early diastolic E and late diastolic A), annular velocity as measured by tissue doppler, e' (septal annular or average of septal and lateral) and ratio of E and e’.

Diastolic dysfunction occurs early in AS. Diastolic dysfunction and elevation of LV end diastolic pressure (LVEDP) could explain exertional dyspnoea in patients with severe AS (45,46). The E/e' ratio correlates well with mean pulmonary capillary wedge pressure measured invasively and is a good echocardiographic correlate of LVEDP (47). An E/e' ratio ≥13 is able to identify patients with a LV end diastolic pressure >15 mmHg with high sensitivity (93 %) and specificity (88 %). Impaired diastolic function is also a predictor of outcome in asymptomatic AS (47). In the study by Lancellotti et al., an E/e' ratio >13.8 was able to identify a subset of patients at greater risk of future events, implying that the presence of severe LV diastolic dysfunction with elevated LV filling pressures is a marker of worse outcome in asymptomatic severe AS (45). In the same study using TDI, the lateral diastolic mitral annular velocity (e') ≤9 cm/s was also able to identify patients at a higher risk of events (45).

LA diameter, area and volume

LA size is an indicator of chronic diastolic dysfunction and has prognostic value in AS (45,48). In AS, LA size increases with worsening diastolic dysfunction and reflects the severity and the chronicity of the increased LA pressure which in turn is a reflection of LVEDP. Atrial enlargement is a marker of progression of valvular disease, and it is directly related to increased ventricular mass in AS patients (49,50) . It also predicts a worse outcome in asymptomatic patients with AS (49,50). LA diameter, was shown to be able to predict progression of symptoms or all-cause mortality in patients with isolated AS and peak aortic pressure gradient ≥50 mmHg. In this study the left atrial diameter was an independent predictor of clinical outcome

(P=0.02) (other predictors were left ventricular end systolic diameter, P = 0.008, left ventricular septum thickness ,P = 0.01) , with no additional value of transaortic gradient (51). The study by Lancellotti et al. showed that an LA indexed area ≥12.4 cm2/m2 in asymptomatic patients could predict future cardiac events (45).

Long axis function

The ventricular wall has subendocardial and subepicardial layers with longitudinally oriented fibres and mid wall with circumferentially oriented fibres (52,53). Elevated end diastolic pressures as a consequence of AS limits perfusion to subendocardium and results in myocyte dysfunction (25,28). As a consequence, one of the first functions to be affected in AS is LV longitudinal function and assessing the LV longitudinal function might be a better way to identify subclinical myocardial dysfunction (9). At this stage of the disease LVEF is still preserved. Studies of LV longitudinal function confirmed that LV longitudinal systolic function is affected in AS, even in asymptomatic patients with preserved LVEF (54). Further, symptomatic patients with preserved LVEF had greater impairment of LV longitudinal systolic function (45,54).

Earlier studies used M-mode assessment of mitral annulus excursion during systole (Mitral annular peak systolic excursion - MAPSE) as a marker of LV long axis function. In the study Takeda S et al., 78 patients with all grades of AS were included (among those with severe AS 23 were symptomatic and 11 were asymptomatic) (54). Compared to controls both septal and lateral MAPSE were lower in AS patients ($p<0.0001$ and $p=0.002$; respectively).Septal MAPSE was

different from controls in all grades of AS ($p<0.05$) whereas lateral MAPSE was significantly different only in severe AS ($p<0.05$). MAPSE was lower in symptomatic than in asymptomatic patients, on both the septal (0.91 v 1.08 cm, $p = 0.05$) and the lateral sides (1.17 v 1.43 cm, $p = 0.04$). There was a linear relation between MAPSE and LV mass index at the septal side ($p < 0.0004$) but not at the lateral side. On multivariate analysis, septal MAPSE was independently related to both LV mass index ($p = 0.001$) and severity of aortic stenosis ($p = 0.002$).

Later, tissue doppler (TDI) techniques replaced M-mode to assess mitral annular excursion. Lancelloti et al. studied systolic and diastolic mitral annular velocities with TDI (54).They followed 126 patients with asymptomatic severe aortic stenosis for a mean of 20 +/- 17 months. In that study, patients with asymptomatic AS who have impaired LV long axis function with peak s' wave $\leq$4.5 cm/s and increased BNP levels $\geq$61 pg/ml are at increased risk of events. They also found late diastolic annular velocity (A velocity) less than 9 cm/sec was associated with excess risk of death, symptoms or surgery.

M-mode and tissue doppler based assessment of mitral valve excursion were limited by the angle-dependency and poor reproducibility. Moreover mitral valve excursion is only a surrogate marker of long-axis function. With the advent of speckle tracking imaging or echocardiography (STI or STE) direct assessment of myocardial function (not just in the longitudinal plane but in different planes) is now possible without reliance on surrogate markers. Using speckle tracking imaging, the deformation of myocardium (lengthening or shortening) can be assessed as strain.

Speckle tracking imaging or echocardiography (STI or STE)

Strain is a measure of deformation, expressed as a fractional change from an object's original dimension (55,56).Within a deforming body, the amount of shortening (or stretch) in the tissue along any axis is called the normal strain and the amount of distortion associated with the sliding of layers relative to each other is called the shear strain (56). Strain rate is the speed at which this deformation occurs. The strain at any point in the tissue is composed of three components of normal strain along three perpendicular axes (x, y, z), and three components of shear strain in one of the three perpendicular planes (xy, xz, and yz). Therefore, for the left ventricle, 3 normal strains (longitudinal, circumferential, and radial) and 3 shear strains (circumferential-longitudinal, circumferential-radial, and longitudinal-radial) can theoretically be used to describe deformation in three dimensions. In addition a combination effect of longitudinal and circumferential deformation, called area strain, can also be assessed. Global longitudinal strain (GLS), global circumferential strain (GCS) ,global area strain (GAS) and global radial strain (GRS) have been measured in this study.

There are two methods for assessing deformation in a tissue. One method is to identify a small volume (sample volume) in the tissue and describe points coming into out of that volume as a function of time ,which yields strain rate (Eulerian or spatial description).The second is to follow a point in the tissue and define its motion as a function of space and time (Lagrangian or material description)(57,58).

Tissue doppler imaging measures strain rate and analyses Eulerian strain which is derived from temporal integration of the strain rate signal. It was introduced several years ago as a method to quantify myocardial function (59). However, tissue Doppler–derived strain has many limitations like angle dependency, noise interference, and significant intra-observer and inter-observer variability (60).

Speckle-tracking echocardiography (STE) is a recent technology which analyses motion by tracking natural acoustic reflections and interference patterns. It tracks user-defined regions containing stable patterns that are described as ‘‘speckles’’ (sometimes called ‘‘markers’’, ‘‘patterns’’, ‘‘features’’, or ‘‘fingerprints’’) (56,57,61).These Speckles are tracked from frame to frame to resolve two-dimensional (2D) and three-dimensional (3D) sequences of tissue motion and deformation. Speckle-tracking technology analyses Lagrangian strain and strain rate can be calculated from the measured strain. In STE the end-diastolic tissue dimension is taken as the reference and represents the unstressed, initial length of the myocardium. So, the radial deformation can be either thickening (positive strain) or thinning (negative strain) of the myocardial wall; circumferential deformation can be either shortening (negative strain) or lengthening (positive strain) of the circumference of myocardial wall in short axis view and longitudinal deformation can be either shortening (negative strain) or lengthening (positive strain) of myocardium in long axis (56). Because there is shortening in longitudinal and circumferential dimensions of LV compared to end-diastole and thickening in radial direction longitudinal (GLS),circumferential (GCS) and area (GAS – which is a combination of GLS and GCS) are negative quantities, whereas radial (GRS) is a

positive quantity. The negative sign only indicates direction and absolute value of the strain is taken into consideration for clinical purposes. Myocardial strain derived from STE is independent of angle and it can be used to analyse the strain in all directions from just apical and short axis views (56,57). STE derived strain has been validated comparing it with other standard research tools for assessing strain in tissues, like sonomicrometry and tagged MRI (62,63). Speckle tracking–derived strain does not require assumption of any chamber morphology or indexing for chamber size .So, it has also been used for studying function of right ventricle and atria whose complex morphology precludes accurate assessment of function with available echocardiographic techniques (64–66). Studies have showed that STE derived strain is highly reproducible with minimal intra-observer and inter-observer variability.

In aortic stenosis, as has already been discussed, patients can remain asymptomatic or minimally symptomatic for prolonged periods even in the presence of severe valvular disease, during which there is progressive adaptation of LV myocardium. The LV ejection fraction, however, remains preserved during this period. Previous studies with tissue doppler echocardiography established that LV systolic longitudinal strain and strain rate are decreased in patients with aortic stenosis even with preserved ejection fraction and improve immediately following aortic valve replacement (67,68). So , STE by virtue of its superior ability to detect longitudinal dysfunction, has the potential to detect subclinical cardiac dysfunction.

Patients with AS may also differ in the regional pattern of myocardial adaptation which cannot be detected by current echocardiographic techniques. STE

derived strain, by assessing deformation in 3 dimensions, can give insight into this variability of adaptation in the LV, which could be of prognostic significance. Studies have shown that STE–derived longitudinal strains are reduced in severe aortic stenosis with preservation of radial and circumferential strains and LV torsion and it has been shown that after surgery strain improves in all directions (69,70).

In one of the first studies of STE measured myocardial strain in AS patients Becker et.al. recruited symptomatic patients scheduled for aortic valve replacement of whom 22 patients had isolated AS (70). They measured radial and circumferential strain preoperatively, immediate postoperative (within 7 days) and six months later. In these patients there was significant improvement in both radial and circumferential strain immediately after surgery and continued to improve till 6 months. There was no healthy control group and the normal range of the values for GRS and GCS were not known at the time of study. They showed that, myocardial deformation parameters were affected by AS and change significantly after aortic valve replacement.

Two studies were done on asymptomatic severe AS patients. Lafitte et al. studied asymptomatic patients with severe AS with echocardiography and exercise testing and compared with 60 normal subjects (69). They studied strain using 2D STE. They found that in comparison to controls, longitudinal strain was significantly lower in AS patients (17.8 vs 21.1, $p < 0.05$). There was no difference with radial or circumferential strain between AS patients and controls. Basal and apical longitudinal strains were separately analysed and found that only basal strain was significantly different in AS patients compared to controls (-12.4 vs -

18.4,p<0.05).There was no difference in apical strain. This regional difference in strain was explained by earlier longitudinal contraction at apex compared to base which ends its longitudinal contraction against a closed aortic valve (71). Strain correlated well with parameters measuring of LV global systolic function but not with measures of AS severity. They calculated a cut-off value of -18% for GLS and -13% for basal strain to predict positive response on exercise stress test (sensitivity/specificity of 68/75% and 77/83% , respectively).Basal strain < |-13%| also significantly predicted clinical events. So despite normal EF, strain was significantly lower in asymptomatic patients with severe AS and there was regional difference in strain with basal segments involved more than the apical segments. In the second study by Zito et.al. fifty two asymptomatic patients with severe AS were prospectively followed for a mean 11 ± 7.5 months and strain was assessed (72). All patients had decreased GLS (-15 ± 4%) but increased circumferential strain (-22 ± 5%).Only the GLS (p = 0.03) and was independently associated with the combined end point. The cut-off for GLS of < |-18%| had 96% sensitivity and 73% specificity for predicting events.

Seventy three patients with severe AS (both symptomatic and asymptomatic were studied by Delgado et.al (73). In these patients with preserved LV EF, strain and strain rate data were compared with data from 40 controls (20 healthy individuals and 20 patients with LV hypertrophy).In contrast to Lafitte's study (discussed above) there was significantly decreased values of LV strain in all three directions - longitudinal, radial and circumferential- compared to controls. At 17 months after AVR, LV strain values significantly improved in all the three directions, whereas

LVEF remained unchanged .The reduction of strain in all directions in contrast to previous study can be explained by inclusion of symptomatic patients, probably indicating that as strain gets affected in all directions symptoms appear.

Arnold CT Ng et al. included a total of 420 patients with aortic sclerosis and all degrees of AS (symptomatic and asymptomatic) (74). Multidirectional strain was assessed by STE. They found a progressive stepwise impairment in longitudinal strain with increasing AS severity. There was no difference in circumferential between aortic sclerosis and mild AS but worsened with moderate to severe AS. Radial strain was decreased only in the presence of severe AS. Compared with asymptomatic patients, symptomatic patients had more impaired strain in longitudinal, circumferential and radial direction.

The following conclusions can be drawn from the above studies (i) strain is reduced in patients with AS before onset of symptoms and with normal EF, (ii) longitudinal strain is the first to be affected and is reduced even in patients with mild AS, (iii) circumferential and radial strain are not affected in milder degrees of AS but are reduced as severity increases, (iv) there is probably a phase when there is compensatory increase in circumferential strain with reduced longitudinal strain, (v) basal longitudinal strain is affected before the apical longitudinal strain and as symptoms appear even apical strain is affected, (vi) reduced longitudinal strain predicts events in asymptomatic severe AS patients and (vii) strain improves in all directions after surgery.

The definition of normal values of left ventricular strain is important for its widespread adaptation into clinical practice. Values of multidirectional strain in healthy individuals have varied in various studies. One cause for concern is the variation in measurements among different vendors due to differences in the software used to measure strain. One large meta-analysis sought to establish normal ranges of strain and identify factors that contribute to reported variations (75). Among 2,597 healthy subjects from 24 studies, reported normal values of GLS varied from 15.9% to 22.1% (mean, 19.7% ; 95% CI, 20.4% to 18.9%). Normal GCS varied from 20.9% to 27.8% (mean, 23.3%; 95% CI, 24.6% to 22.1%) and GRS ranged from 35.1% to 59.0% (mean, 47.3%; 95% CI, 43.6% to 51.0%). There was significant heterogeneity between studies. After analysis, only blood pressure (but not age, gender, equipment vendor or frame rate) was associated with variation in normal GLS values. For GLS they were able to establish normal value as -19.7 ± 0.28 . Similar values for GRS, GCS and GAS from large populations are yet to be published.

Three dimensional echocardiography (3DE)

The earlier technique of 3D reconstruction from images acquired through 2D echocardiography has been replaced by the development of real time 3D echocardiographic (RT3DE) systems which can acquire images in three dimensions (76). RT3D echocardiography uses a transducer with ultrasound elements arranged in a grid fashion and acquires a pyramidal volume. Current RT3D systems use matrix-array transducer technology and typically contain more than 3000 imaging elements and improvements in transducer technology have resulted in smaller transducer footprint, better contrast and greater sensitivity and penetration. Three

acquisition modes are usually present in RT3D systems. Namely, real time (narrow), zoom (magnified) and wide angle. Once a 3D image is acquired, it can be sliced or "cropped" and can be manipulated to appropriately align the cardiac structures to visualize the desired structure or view within the acquired pyramidal volume (76).

In aortic stenosis the potential uses of RT3DE can be for calculating LVOT area which can be used to calculate AVA by continuity equation .RT3DE can be used for direct tracing of AVA (Planimetry) and assessing aortic valve morphology and measure LV function by 3D ejection fraction and calculating LV mass. In this study, RT3DE was used in acquiring images for measuring strain. A single full volume acquisition from apical view can be used for analysing strain by STE in all three directions (GLS,GCS and GRS) and also global area strain (GAS).In GE vivid E9 ultrasound system (used in this study), compared to 2D STE , three dimensional echocardiography has some advantages (77,78). First, only one image acquisition is required. Second, unlike 2D images there is no overlap in myocardial segments which happens with acquisition in multiple views and generating bull's eye diagram. Third, all four strains can be analysed together and bull's eye diagram generated for each strain separately. Fourth, off plane movement, which is a problem with 2D-STE, is not there in 3D acquisition. The major disadvantage is the larger foot print of the transducer and resultant poor image quality in some patients with poor echocardiographic windows.

Plasma N-Terminal Pro brain natriuretic peptide (NT-ProBNP)

Brain (B-type) natriuretic peptide (BNP) is a 32-amino acid neurohormone synthesized in the ventricular myocardium. The first peptide formed in the synthesis of BNP is pre–prohormone BNP (Pre-ProBNP), a 134 amino acid peptide that is synthesized in the myocytes and cleaved to the prohormone BNP (ProBNP) of 108 amino acids. ProBNP is cleaved by a circulating endoprotease, termed corin, into two polypeptides: the inactive N terminal pro-BNP (NT-ProBNP), 76 amino acids in length, and BNP, a bioactive peptide 32 amino acids in length (79–81).

BNP causes natriuresis and diuresis, arterial vasodilation and antagonizes renin–angiotensin–aldosterone system and the sympathetic nervous system. Unlike BNP, NT-ProBNP has no biological activity or receptors (82). As a result, its half-life is longer (120 min vs 20 min), it circulates in plasma in higher concentrations and it is less influenced by acute changes in the rate of secretion. Less than 5% of BNP is cleared via kidneys, whereas almost all of NT-pro-BNP is excreted by kidney making NT-ProBNP less reliable in the setting of renal dysfunction. BNP and NT-ProBNP are released from cardiac myocytes in response to increases in ventricular wall stress (82,83). In AS increase in LV pressure and therefore an increase in LV wall stress contributes to elevation of natriuretic peptides observed in patients with AS (82,83).

In aortic valve stenosis structural and functional changes in the ventricular myocardium precede symptom development by a long duration and trigger natriuretic peptide release (84–86). In all studies which included both BNP and NT-ProBNP, the latter has appeared to be more powerful predictor of clinical endpoints.

One of the major advantages of NT-ProBNP is that it is simple, relatively inexpensive, reproducible and not operator dependent (87). Whereas, echocardiography requires trained and experienced sonographers with meticulous attention to the technical details (87).

In the study by Gerber IL et al., where 74 patients with moderate to severe aortic stenosis (29 asymptomatic and 45 symptomatic) were compared to 100 controls (87).They studied NT-ProBNP, ANP and BNP and found similar results with all three. Log transformed NT-ProBNP levels correlated moderately with the aortic valve area (r=0.57), LV mass index (r0.59), right ventricular systolic pressure (r0.59, p 0.05 for all comparisons).After adjustment for other confounding variables like age, sex, creatinine clearance and EF, the NT-ProBNP levels were higher in symptomatic patients compared to asymptomatic patients (mean 1.74 times higher;P0.014). NT-ProBNP was higher in patients with NYHA class II symptoms than those with NYHA class I symptoms (median- 105 pmol/l (889.83 pg/ml) vs 34 pmol/l (288.14); p<0.0001). NT-ProBNP levels increased with increasing severity of AS. But there was no statistically significant difference in NT-ProBNP levels between patients with normal diastolic function, Grade I and Grade II diastolic dysfunction. When ROC was analysed NT-ProBNP had excellent AUC of 0.84 with the best cut-off of 60 pmol/l (508.48 pg/ml) for predicting symptomatic status with a sensitivity of of 78% and specificity of 79%.There was no difference in NT-ProBNP levels in patients with and without angina after adjustment for dyspnea level.

The same group followed 29 asymptomatic patients from the initial cohort with for 18 months (88). Overall 8 of the 29 patients developed symptoms. Patients were

classified to have elevated or normal NT-ProBNP at baseline based on a cut-off value of 50 pmol/L (423.73 pg/ml). More patients in elevated NT-ProBNP group (55%) developed symptoms compared to patients with normal NT-ProBNP (11%) with a OR of 9.6 (95% CI 2 to 64, p 0.02). After adjusting for age, peak aortic jet velocity, and the ejection fraction at baseline, the OR was 13 (95% CI 1 to 164, p 0.05). Based on serial peptide measurements, they concluded that in many patients there is a time lag of 1 year between increase in NT–ProBNP above the normal range and development of symptoms.

The study of Bergler-Klein et al. included 130 patients (87 symptomatic and 43 asymptomatic) with severe AS and followed them for a mean 370 ± 150 days (12).In contrast to Gerber et.al there was no significant difference in NT-ProBNP between NYHA class I and NYHA class II patients. This was explained by inclusion of only severe AS patients in this study as compared to inclusion of moderate AS patients in Gerber et.al. study. They showed that patients with severe AS and NT-ProBNP levels <80 pmol/l (677.97 pg/ml) were unlikely to develop symptoms during a nine months follow-up period, having a symptom free survival 88% at 9 months and 69% at 12 months.43 However, patients with NT-ProBNP above these cut-off value had a symptom free survival of 35% at 9 months and 12% at 12 months. Seventy nine patients eventually underwent surgery and NT-ProBNP independently predicted postoperative outcome.

There are three other studies which used BNP instead of NT-ProBNP, but the results were similar. In the study by Lim et al. which included 70 patients with severe AS (17 asymptomatic and 53 symptomatic) and followed them for a mean of 308

days, high BNP levels were significantly associated with poor outcome in asymptomatic patients with severe AS and BNP levels were highly accurate to predict symptomatic status in these patients (89). Lanceolloti et al. studied 126 patients with asymptomatic severe aortic stenosis over a period of 20 months +/- 17 months (45). In that study, patients with asymptomatic AS who had increased BNP levels were at increased risk of events. Monin et al. developed a risk score after prospectively following 107 asymptomatic patients with moderate to severe AS (90).They further validated the score in another cohort of 107 patients. They showed that the combination of serum BNP levels with female sex and Vmax (Monin's score = [peak velocity (m/s)×2]+ [ln BNP (pg/ml) ×1.5]+ 1.5 (if female sex)) can help discriminate between patients who will experience cardiac events during a two year follow-up and those who will not.46 For score values less than 11, a low event rate was found (<10 %), whereas score values above 16 were associated with higher event rate (>75 %).

From the above studies it can be concluded that (i) NT-ProBNP levels increase with increasing severity of AS,(ii) levels are significantly higher in symptomatic patients compared to asymptomatic patients ,(iii) levels increase modestly from mild symptoms to moderate symptoms and markedly from moderate to severe symptoms, (iv) levels significantly predict events and (v) NT-ProBNP levels are independent predictor of postoperative outcome.

Material and methods

Setting:

The study was carried out in the Christian Medical College, Vellore (CMC). Patients were recruited from

1) Patients visiting outpatient services in the Department of Cardiology and

2) Patients admitted in Cardiology and Cardiothoracic wards.

The study was started from January 2014 and was continued till January 2015. Data collection was done during the patient's visit to echocardiography lab as a part of evaluation and during patient's stay in the hospital, by the principal investigator.

Participants:

Inclusion criteria:

1) Adult patients (> 18 years of age) with moderate to severe valvular aortic stenosis diagnosed by echocardiogram were eligible for the study.

Exclusion criteria:

1) Concomitant mitral valve disease - more than mild mitral stenosis or regurgitation as defined by current echocardiographic criteria

2) Significant aortic incompetence - more than mild associated AR as defined by current echocardiographic criteria

3) Past history of acute coronary syndrome or regional wall motion abnormality of LV on echocardiography

4) Past coronary or valvular surgery or percutaneous intervention

5) Patients other than sinus rhythm

6) LV systolic dysfunction defined as EF< 50%

7) Renal dysfunction - estimated GFR less than 30ml/min or patients on dialysis.

8) Pregnancy

Sample size:

This study looks at the speckle tracking imaging derived left ventricular strain and NT-ProBNP in patients with aortic stenosis. The resource limiting variable in the present study was the cost of NT-ProBNP. So sample size calculation was done based on the data about NT-ProBNP in predicting symptomatic status and outcomes. Based on the data from Ivor Gerber et.al (Circulation 2003; 107:1884-18L (949.15 pg/ml) in asymptomatic and symptomatic patients, respectively. The interquartile ranges were 16 to 58 pmol/l (135.59 to 491.53 pg/ml) and 71 to 93 pmol/l (601.70 to 788.14 pg/ml)in asymptomatic and symptomatic patients, 90) (87) the median plasma NT-Pro BNP levels were 33 pmol/L (279.66 pg/ml) and 112 pmol/respectively. The estimated SD was 28 pmol/l (237.29 pg/ml) in asymptomatic group and 78 pmol/l (661.02 pg/ml) in symptomatic group. Keeping alpha error at 5% and beta error at 10%, the sample size needed was calculated as 12 symptomatic and 12 asymptomatic patients. As we derived SD from the range of values provided in the study, there

would have been some over or underestimation in standard deviation. Moreover, as this is an institution based study, the symptomatic patients are likely to be much more common than asymptomatic patients. Considering these issues, we studied patients presenting between Jan/2014 and Jan/2015 (up to 50 patients), anticipating 20-25 patients in each group. We were able to recruit 33 patients in the given time period.

In the present study plasma levels of NT-ProBNP were compared across the groups of patients with aortic stenosis. However, the mean and distribution of serum NT-ProBNP in normal Indian population is not known. To make the comparisons meaningful, an estimate of the normal value for the NT-ProBNP in our population was required. So a control population of 10 subjects provided an estimate of the normal value. Ten age and sex matched controls were selected from healthy relatives of patients coming to this hospital.

Data sources and measurements:

(I) History, physical examination and echocardiography was done by the principle investigator and data was collected with the help of a raw data collection sheet and Echocardiography pro forma.

(II) **Echocardiography**

All patients underwent echocardiography with GE vivid 9E ultrasound system. Two transducers were used.

M5S-D: a phased array transducer with a frequency range of 1.5-4.6 was used for M-mode, 2D and doppler imaging.

4V-D: a volume phased array transducer with a frequency range of 1.5–4.0 MHz was used for full volume acquisition.

A) Two dimensional and doppler echocardiography

Two and three dimensional grey scaled images were stored in cineloop and analysed offline. All standard measurements were done according to recommendations by ASE/ESE. End-diastolic and systolic interventricular septal thickness (IVSd and IVSs), LV internal diameter (LVEDD and LVESD) and posterior wall thickness (PWd and PWs) were measured in 2D in PLAX view. LV mass was calculated automatically by the system using Devereux formula and indexed by the body surface area to derive LV mass index. Left ventricular outflow tract (LVOT) diameter was measured in PLAX view parallel to the aortic annulus at the junction of bright and dark regions indicating septal endocardial and aortic annular tissue junction (as recommended by ASE /ESE). Aortic valve was imaged in SAX view at the level of the valve. The LV end-diastolic and end-systolic volumes were measured from the apical two- and four-chamber views, and LVEF was calculated using the biplane Simpson's method. Similar method was used to calculated left atrial volume and was indexed to BSA. Left ventricular diastolic function was evaluated using transmitral early (E-wave) and late (A-wave) diastolic inflow velocities, the E/A ratio and the deceleration time obtained from the pulsed-wave Doppler recordings. Tissue Doppler echocardiography was performed and peak early diastolic mitral annular velocity (e') was measured on the septal side and lateral side and average was calculated. E/e' was calculated by using medial e' and average e' (Both correlated excellently. So E/e' using medial e' was analysed in this

study). Aortic valve flow velocities were measured with continuous doppler in apical 5-chamber (A5C), apical 3-chamber (A3C or APLAX) and when satisfactory 2D images were feasible doppler velocities were measured from suprasternal and right parasternal views. The maximum velocity in any view was taken as the aortic valve Vmax and the doppler spectral pattern was traced to get mean velocity and aortic valve velocity time integral (AV VTI).Peak and mean gradient was automatically calculated by the system from the measured velocities using modified Bernoulli equation ($4v^2$). Pulse wave doppler of left ventricular outflow tract (LVOT) was acquired from apical 5 chamber view with the sample volume moved proximally from the level of the aortic annulus until a clear signal was obtained, usually 0.5–1 cm below the valve. The spectral pattern was traced to measure LVOT VTI. Aortic valve area was calculated by the online software by continuity equation using the above data. For LVOT diameter, AV VTI and LVOT VTI, an average of three measurements was taken.

B) Speckle tracking imaging

Vivid 9E system has two software tools available to assess LV strain (77). Longitudinal strain can be assessed using automated functional imaging (AFI) software and strain in all directions (Longitudinal, circumferential, area and radial) can be calculated by using 3D images acquired with a 4V-D transducer and analysed with 4D automated LV quantification (4D-auto LVQ) software.

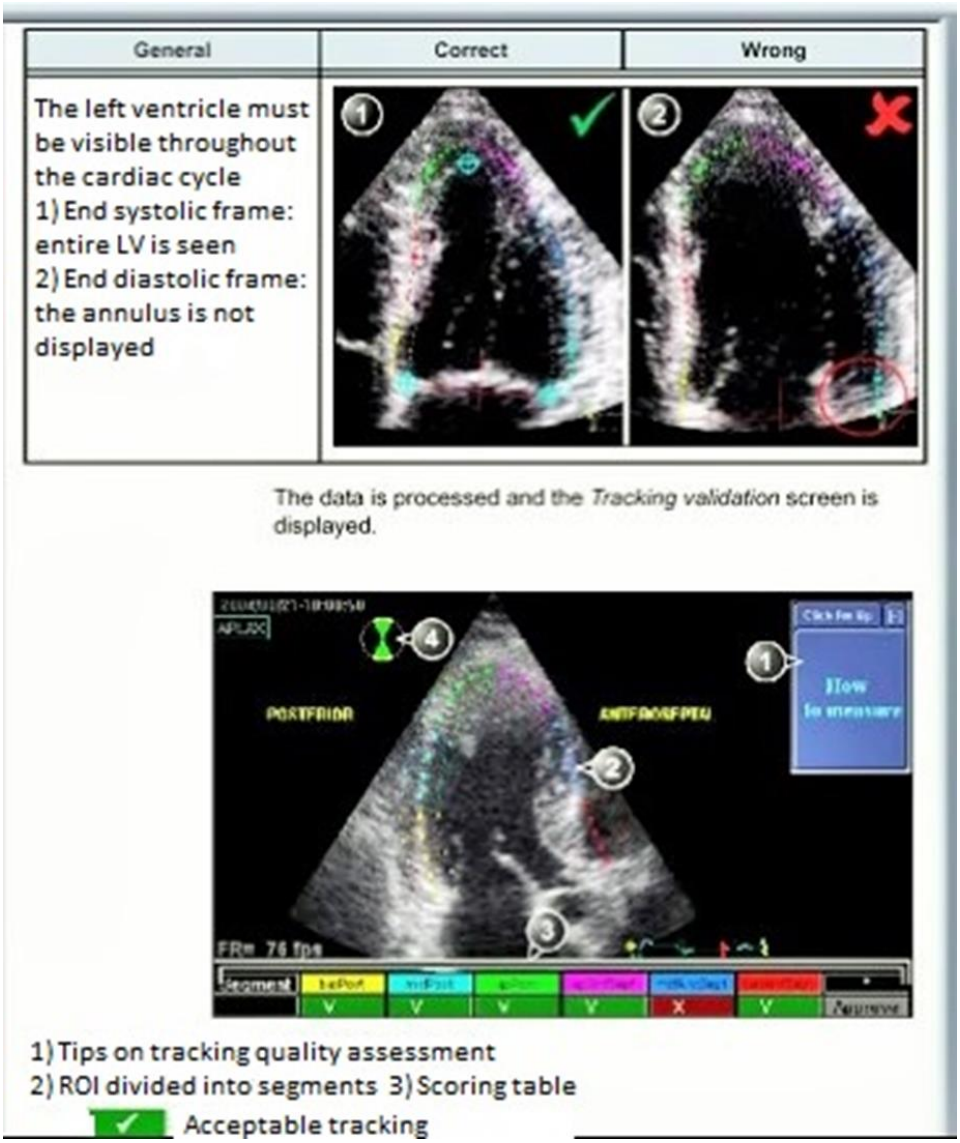
(i) **Automated functional imaging (AFI)**

AFI is a software tool used for regional assessment of 2D longitudinal strain which calculates myocardial deformation based on 2D grey scale loops. AFI is performed in apical views in the following order: apical long-axis, 4-chamber and 2-chamber views following an onscreen guided workflow (Fig.1) .The apical views

Fig. 1.

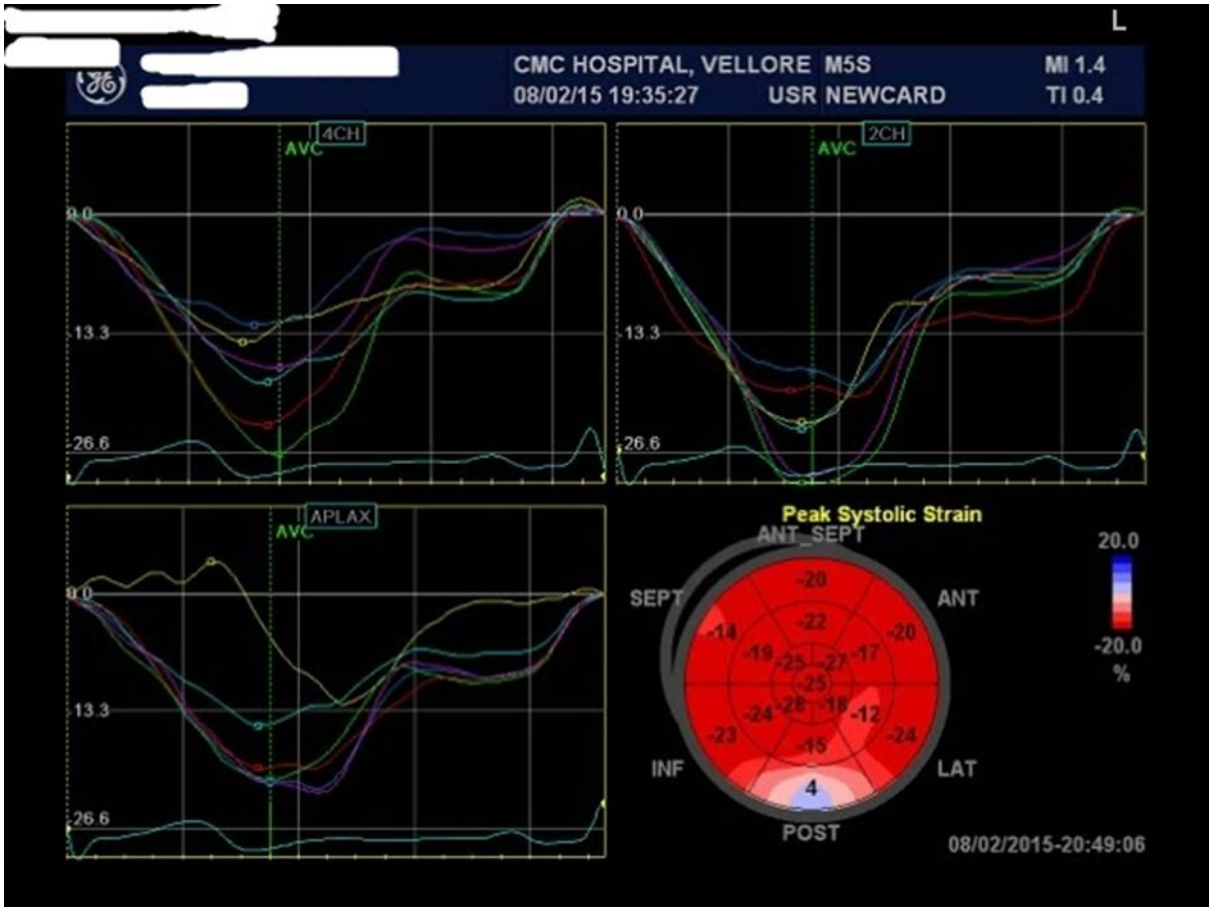
may be acquired sequentially in 2D mode or simultaneously in tri-plane with a 3D transducer. After acquisition of the grey scale loops analysis can be done off-line. The frame rate should be 40-80 fps (higher if HR is high) and care should to be taken such that the entire myocardium is visible. It is important to acquire all apical views sequentially to get similar heart rate in all three views (77,91).In each view, region of interest (ROI) is defined by placing 2 points at the base near the mitral annulus and one at apex following the order shown onscreen and these points trigger the automatic process by which the software detects the borders (Fig.2) . The ROI

Fig. 2



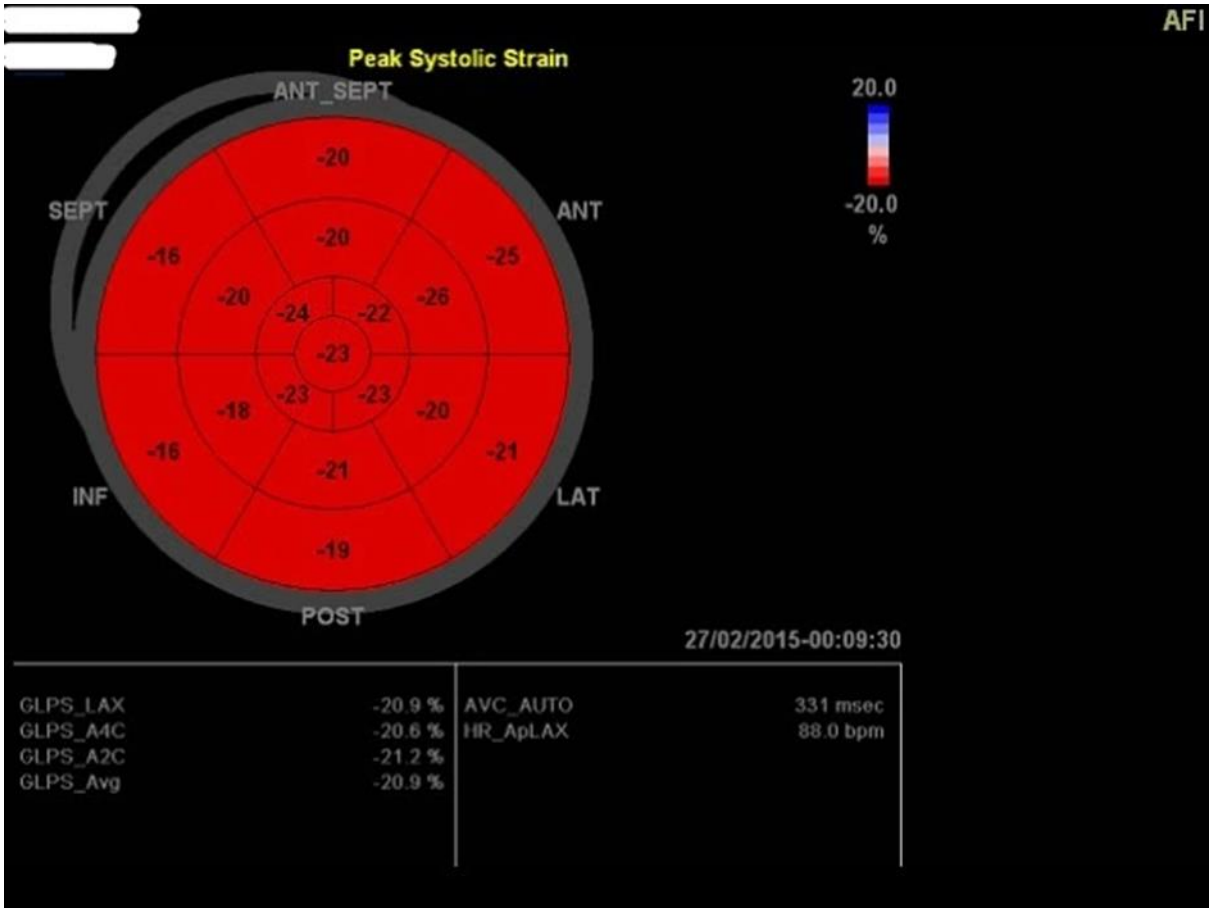
can be manually adjusted by moving the borders. The system shows preliminary images and the operator can assess tracking quality and if the tracking is poor, analysis for that view is repeated after readjusting the endocardial tracing until a better tracking is achieved. Inadequately tracked segments are automatically excluded from analysis (Fig.2). If more than 2 segments are not adequately tracked, longitudinal strain from that view is not calculated. The process is repeated for all three apical views. At the end, the longitudinal strains for each individual segment are displayed as curves as a function of time (Fig. 3). At the end, the average of peak systolic longitudinal strain of individual LV segments in each view (GLS_LAX,

Fig. 3



GLS_A4C,GLS_A2C) and average of all views (GLS_Avg) is given. The results of all 3 planes are also combined into a single colour coded bull's-eye summary (Fig.4) , which presents the peak systolic longitudinal strain of each segment along with a global longitudinal strain value for the LV (77,91).

Fig.4

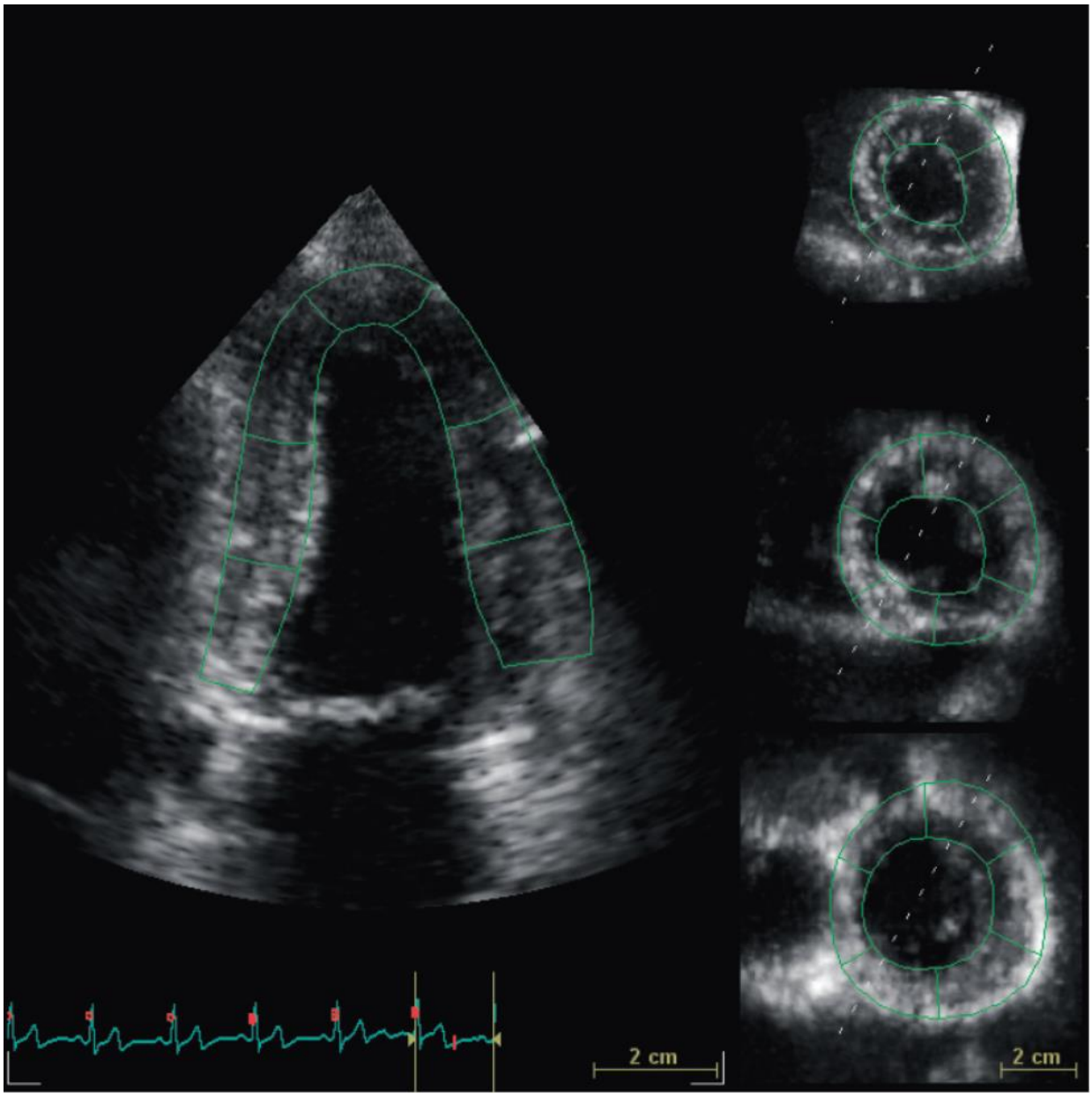


(ii) 4D- automated LV quantification (4D-Auto LVQ)

4D-Auto LVQ is a post-processing software tool for analysing LV volumes, LV mass and strain using a single 3D image acquired with a 4V-D transducer from apical view (77,78). Region of interest (ROI) is defined manually by placing 3 points on the endocardial border , two at the base and one at apex .The system automatically

demarcates endocardial and epicardial borders, which can be adjusted manually (Fig. 5). As a first step, end-systolic and end-diastolic frames are used to calculate LV volumes and 3D EF. Second step calculates LV mass by following similar method.

Fig. 5



4D Strain analysis is integrated as the last step in the 4D Auto Left Ventricular Quantification tool. The meshes (segmented regions between endocardial border and epicardial borders) created for measuring LV volume and LV mass are re-used for the 4D Strain ROI (Fig.5). The 4D Strain ROI is automatically generated in the end-systolic frame and is built up from an endocardial and an epicardial mesh already used for assessing 3D EF and LV mass. The user can correct the ROI shape by placing attractor points to pull the nearby ROI border towards where the user wishes it to go. The frame rate has to be above a minimum level of at least 40% of the heart rate and care should be taken to check proper tracking (78). The user interface allows the user to reject segments with sub-optimal tracking. The

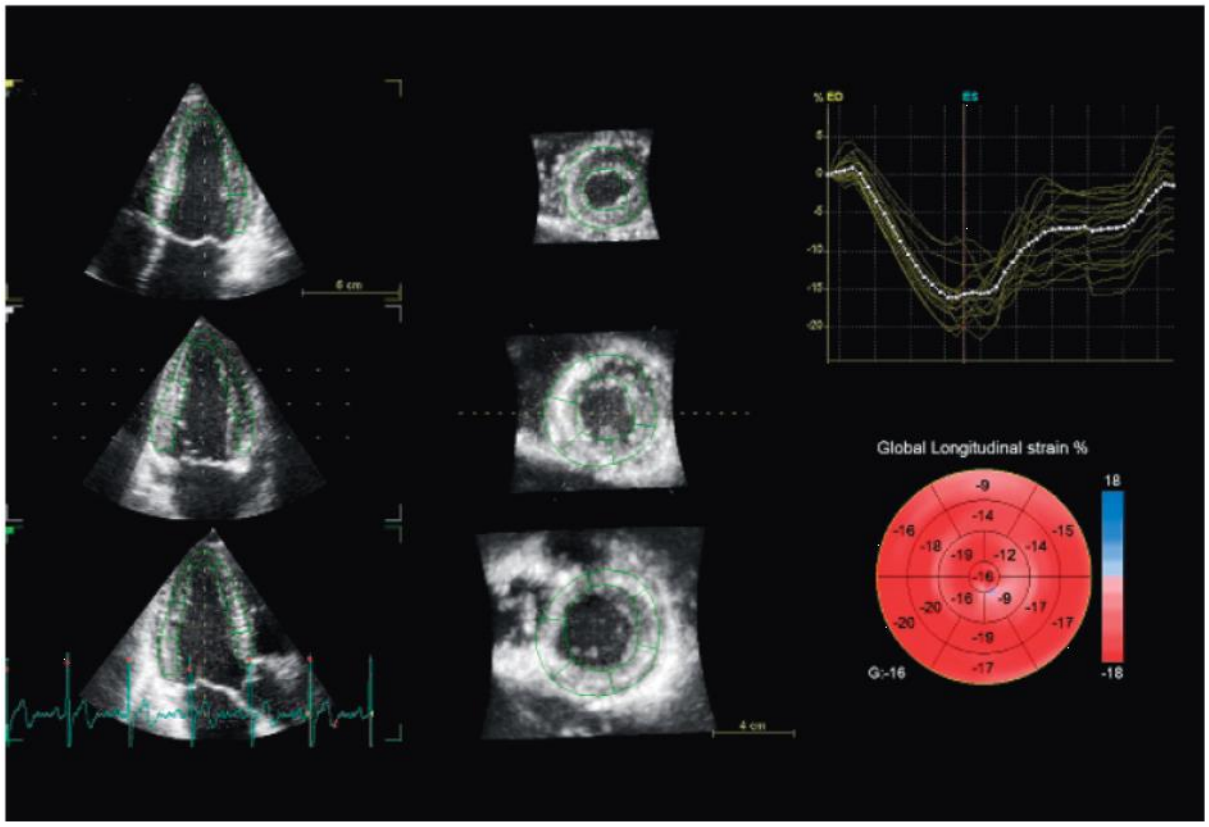


Fig.6

results for these segments will then be removed. Furthermore, global strain values will not take these into the calculation and if more than three segments are rejected, global strain values will not be calculated. From the tracking results, 4D Strain derives longitudinal, circumferential, area and radial strain. The calculated parameters are presented in various ways, including a colour coded bull's eye plot (Fig.6). The ventricle is divided into 17 segments, and the instantaneous strain throughout the cardiac cycle is displayed numerically and using colour coding in the bull's eye plot . Care should be taken when comparing 2D AFI and 4D Strain, as the strain values in 2D AFI are the peak systolic values, including positive peaks, while in 4D Strain it is the strain values from the current frame, typically the end systolic frame, that are used. Bull's eye plots for GCS, GAS and GRS can also be generated after GLS is done (Fig.7).

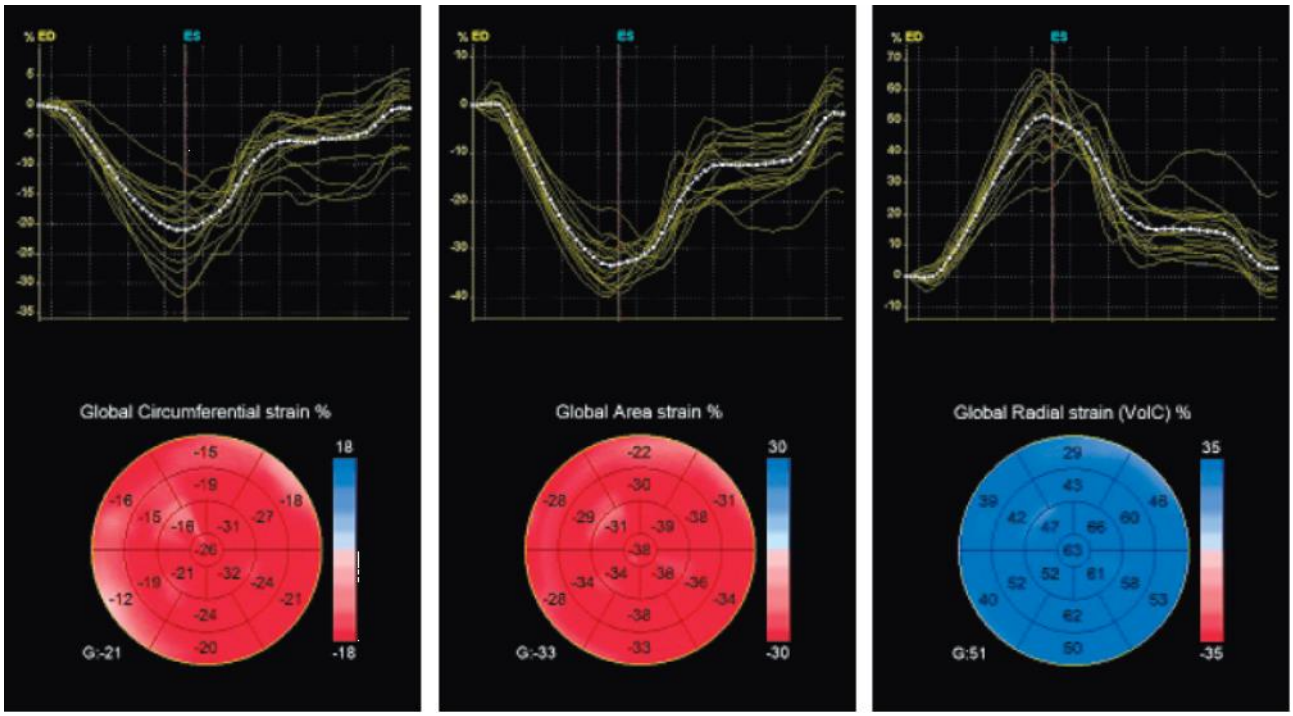


Fig. 7

In this study as 2D images from apical vies were consistently of good quality 2D-STE derived GLS was used for analysis. GCS,GAS and GRS were measured using 4D-Auto LVQ.

3) Plasma NT-ProBNP

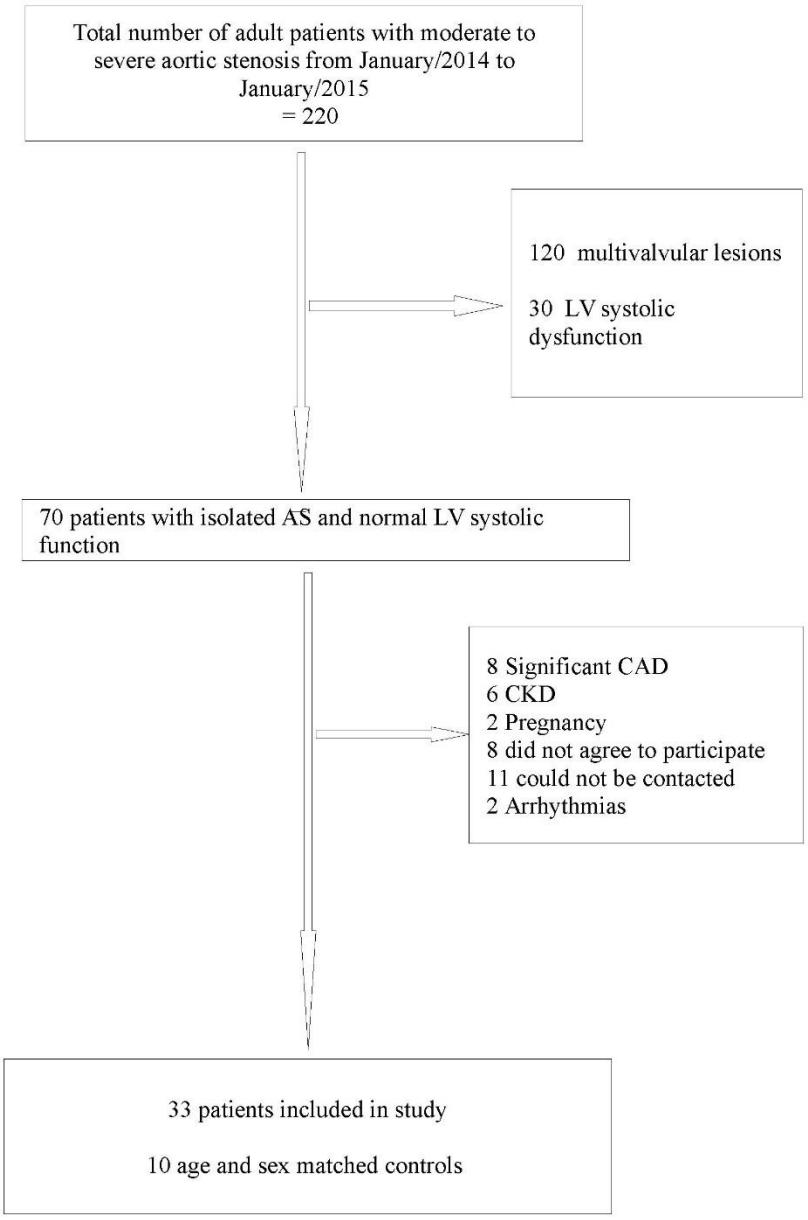
The sample was collected at the entry into study after consent. Two to three millilitres of blood was collected in a lithium heparin tube after patient in recumbent position for at least 1 hour (this time was utilized for interview and echocardiography) and was sent to the department of Biochemistry for quantitative analysis of plasma NT-ProBNP .NT-ProBNP assay is an automated, double incubation sandwich assay (Roche diagnostics). In the first incubation, patient specimen NT-ProBNP antigen reacts with biotinylated monoclonal sheep NT-ProBNP antibody and monoclonal NT-Pro BNP antibody labelled with ruthenium complex. During the second incubation, streptavidin labelled micro particles are added. This results in a complex bound to the solid phase via biotin-streptavidin interaction. The resulting reaction mixture is aspirated into the measuring cell .The micro particles are magnetically captured onto the surface of an electrode. Unbound substances are washed away. Then a voltage is then applied to the electrode, which induces chemiluminescent emission from bound ruthenium that is measured by a photomultiplier (Electrochemiluminiscense immuno assay - ECLIA). Results are calculated by comparing this measurement against the calibration curve.(Kit from Roche Diagnostics Corporation. Instrument COBAS E 411).

Statistical methods:

Continuous variables were scrutinized for normal distribution using histograms and box-whisker plot. They were expressed mostly as mean ± standard deviation. When the data was skewed, median and interquartile ranges were reported. As some variables were skewed and number in each subgroup was small, non-parametric tests were used. For comparison between two groups Mann-Whitney U test and between more than two groups Kruskal-Wallis test war used. Categorical data were expressed as numbers and percentages and comparison between groups was done using Fisher's exact test. For correlation between continuous variables Pearson's correlation coefficient was used.

All data was entered on Microsoft Excel 2010 spreadsheets and then Statistical analysis was performed using SPSS software version 16.0. A P-value 0.05 was considered statistically significant.

STUDY ALGORITHM

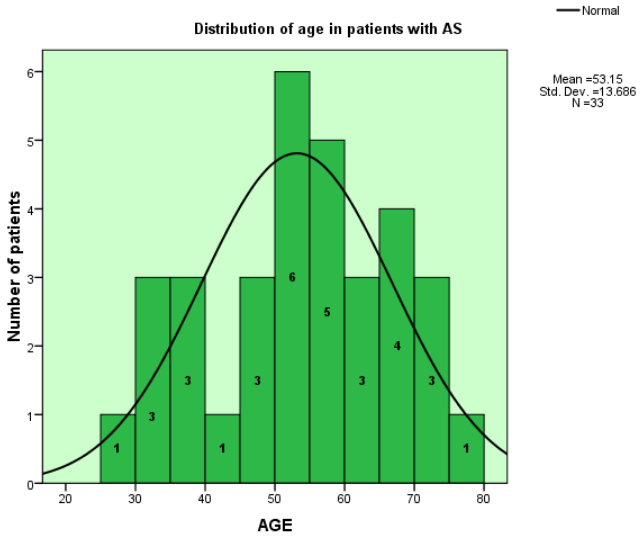


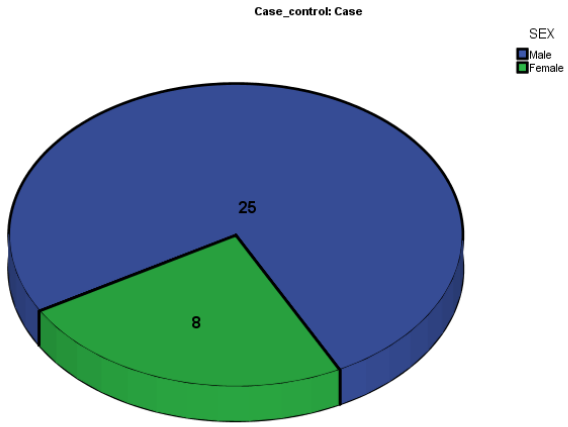
Results

Thirty three patients with aortic stenosis were recruited during the period. Ten age and sex matched healthy subjects were taken as controls.

Age and sex:

The mean age of the patients was 53.2 years with a standard deviation of 13.7 years, median of 54 years and a range 26-77 years .The patients comprised of 25 (76%) males and 8 (24%) females. Mean age of control population was 46.6 ± 16.2 years with a median of 47.5 and a range of 26 - 74 years. There were 7 (70%) males and 3 (30%) females in the control group. Symptomatic patients were older compared to symptomatic patients (57.2 vs 46.1 years).





Associated conditions:

Seven (21%) of the patients had type 2 diabetes mellitus. Ten (37%) patients had hypertension and twelve (36%) patients were either smokers or ex-smokers. They did not differ significantly between asymptomatic and symptomatic patients.

Table.1 gives the distribution of clinical variables in the patient and control population.

	Total patients (n = 33)	Moderate AS (n = 6)	Severe AS (n = 27)	Controls (n= 10)
Age (yrs)	**53.2 ± 13.7**	47.3 ± 11.2	54.4 ± 14.0	**46.6 ± 16.2**
Sex				
Male n(%)	**25 (76)**	4	21	**7 (70)**
Female n(%)	**8 (24)**	2	6	**3 (30)**
BSA (m^2)	**1.71 ± 0.18**	1.68 ± 0.23	1.72 ± 0.18	**1.75 ± 0.17**
Systolic BP (mmHg)	**126.6 ± 20.4**	116.8 ± 14.2	128.8 ± 21.2	**126.2 ± 9.5**
Diastolic BP (mmHg)	**74.9 ± 9.1**	75.8 ± 11.8	74.7 ± 8.7	**76.2 ± 4.4**
Heart rate (bpm)	**77.6 ± 13.4**	74.5 ± 8.6	78.3 ± 14.3	**76.0 ± 15.5**
Creatinine clearance (ml/min)	**85.1 ± 19.9**	95.2 ± 19.1	82.9 ± 19.7	**90.3 ± 28.8**
Hypertension n (%)	**10 (30)**	0	10	-
Diabetes mellitus n (%)	**7 (21)**	1	6	-
Smoking n (%)	**12(36)**	1	11	-
Symptoms				
Dyspnea n(%)	**21 (64)**	1	20	-
Angina n(%)	**6 (18)**	1	5	-
Syncope n(%)	**1 (3)**	0	1	-

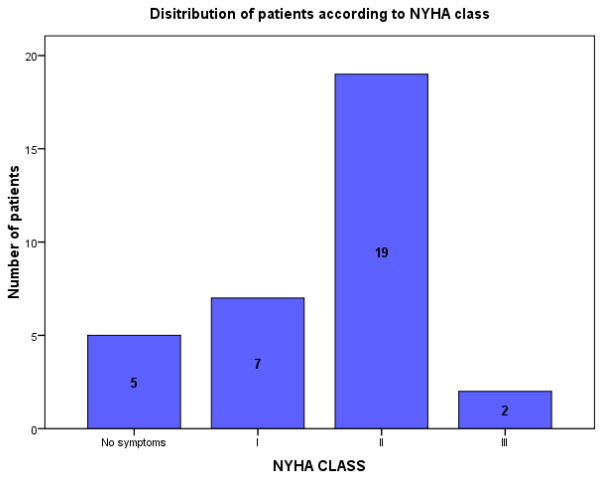
* Values are mean ± standard deviation

Creatinine clearance (eGFR):

Mean creatinine clearance was 85 .13 ± 19.9 ml/min in the patients and was similar in the controls with a mean of 90.30 ± 28.80 ml/min. Patients with eGFR less than 30ml/min were excluded from this study. There was no significant difference in creatinine clearance between controls, symptomatic and asymptomatic patients.

Symptoms:

Minimally symptomatic (NYHA class I) and asymptomatic patients were grouped together as asymptomatic for the purpose of analysis and patients with NYHA class II or more symptoms were considered symptomatic. Twenty one patients (64%) were symptomatic and 12 patients (36%) were asymptomatic (5 patients with moderate AS and 7 patients with severe AS). Dyspnea was the most common symptom with all symptomatic patients having dyspnea as one of their symptoms. Nineteen patients (58%) had NYHA class II dyspnea and 2 patients (6%) had NYHA class III dyspnea. None of the patients had NYHA class IV symptoms.

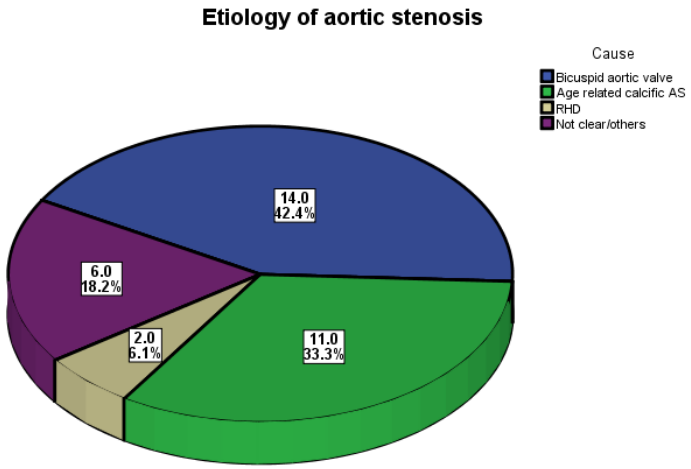


Angina was an additional symptom in 6 (18%) of the symptomatic patients with dyspnea

and all except one had CCS class II angina. One patient had CCS class III angina. Only one patient had syncope, who also had severe dyspnea and angina (class III) .Six of the symptomatic patients also complained of exertional palpitations. None of the patients had features of fluid overload.

Etiology of aortic stenosis:

Echocardiographically aortic valve was visualized in short axis to assess the morphology. The etiology was considered definite bicuspid aortic valve (BAV) if unequivocally visualized. When three cusps were visualized and calcification at the base was seen in appropriately aged patient, it was considered age related calcific AS. Rheumatic involvement of mitral valve along with commissural fusion in AV was considered rheumatic AS (RHD). When the patient has undergone surgery (as was the case in 3 patients) the pathological diagnosis was considered definitive for the etiology. When the valve leaflets are not properly visualized or the etiology was not clear, it was labelled as 'uncertain/others'. Two of these patients were less than 50 years with tri-leaflet aortic valve and minimal calcification, probably congenital aortic stenosis with three leaflets.



BAV was the most common cause with 14 (42%) patients. Eleven (33%) patients had age related calcific AS and 2(6%) patients had rheumatic heart disease with isolated aortic stenosis (they had thickened and deformed mitral valve leaflets with mild or no MS or MR).In 6 (18%) patients the cause was uncertain.

Echocardiographic variables:

Table 2. Shows the echocardiographic variables in patients and controls

	Total patients (n=33)	Moderate AS (n= 6)	Severe AS (n=27)	Controls (n=10)
IVST (cm)	1.47 ± 0.31	1.22 ± 0.44	1.52 ± 0.24	0.96 ± 0.17
PWT (cm)	1.43 ± 0.30	1.18 ± 0.47	1.49 ± 0.22	0.96 ± 0.14
LV EDD (cm)	4.15 ± 0.57	4.18 ± 0.76	4.14 ± 2.75	4.04 ± 0.50
LV ESD (cm)	2.26 ± 0.56	2.77 ± 0.69	2.75 ± 0.54	2.75 ± 0.36
LV EDV (ml)	88.94 ± 33.11	84.00 ± 22.67	90.04 ± 35.26	73.90 ± 18.63
LV ESV (ml)	34.73 ± 16.00	30.33 ± 12.03	35.70 ± 16.80	27.90 ± 7.48
LV EF (%)	61.61 ± 6.17	64.50 ± 7.89	60.96 ± 5.71	62.40 ± 3.69
LA volume index (ml/m^2)	26.5 ± 9.48	27.58 ± 15.02	26.26 ± 8.18	19.26 ± 4.68
LV mass index (ml/m^2)	129.76 ± 51.16	113.28 ± 80.88	133.42 ± 43.44	64.84 ± 15.72
LVOT diameter (cm)	2.07 ± 0.29	2.08 ± 0.19	2.06 ± 0.31	2.05 ± 0.21

* Values are mean ± standard deviation

Table 2. Continuation

	Total patients (n=33)	Moderate AS (n= 6)	Severe AS (n=27)	Controls (n=10)
Mitral valve				
E/A	**0.92 ± 0.32**	1.08 ± 0.17	0.88 ± 0.34	**1.35 ± 0.34**
Deceleration time (msec)	**209.58 ±74.80**	211.67 ±12.9	209 ±60.60	**186.00 ±20.67**
Medial e'	**0.05 ± 0.02**	0.07 ± 0.02	0.04 ± 0.01	**0.10 ± 0.02**
E/e'	**19.26 ± 9.91**	1.08 ± 0.17	19.81 ± 9.88	**8.78 ± 2.07**
AV Vmax (m/sec)	**4.39 ± 0.73**	3.43 ± 0.20	4.60 ± 0.62	**1.12 ± 0.14**
AV mean gradient (mmHg)	**49.68 ±18.41**	28.97 ±2.50	54.29 ±17.17	**2.62 ±0.51**
AV VTI (cm)	**98.06 ±24.54**	74.15 ±4.60	103.37 ±24.00	**23.88 ±3.36**
LVOT VTI (cm)	**23.79 ± 5.00**	26.85 ± 4.90	23.11 ± 4.85	**22.63 ± 3.57**
AVA index (cm^2/m^2)	**0.50 ± 0.15**	0.73 ± 0.14	0.45 ± 0.09	**1.8 ± 0.32**

* Values are mean ± standard deviation

Aortic stenosis severity:

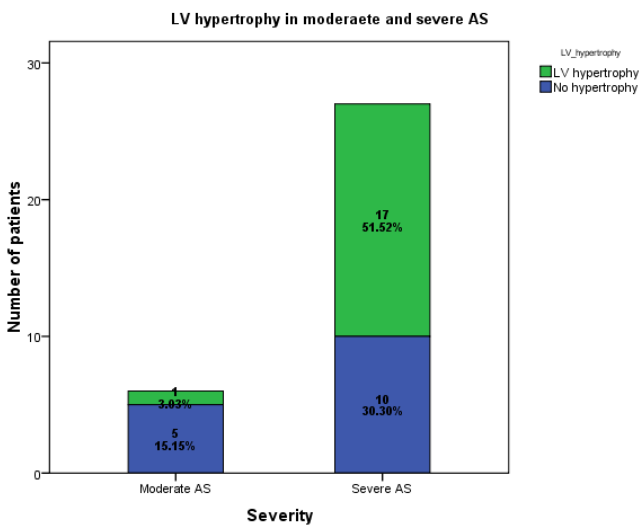
Twenty seven (82%) patients were classified as severe aortic stenosis (AVA < 1.0cm) and 6 (18%) had moderate AS. Two patients had peak velocity and mean gradients just below the cut-off values (AV peak velocities -3.88 and 3.80 m/sec, AV mean gradient - 36.85 and 36.33 mmHg) but AVA less than 1cm^2 by continuity equation (AVA -0.7 and 0.6 cm^2).Both of them had small LV volumes with stroke volume index of < 35ml/m^2 (SVi - 30.6 and 33.7 ml/m^2).These two patients had low-flow, low-gradient AS with preserved LV EF.

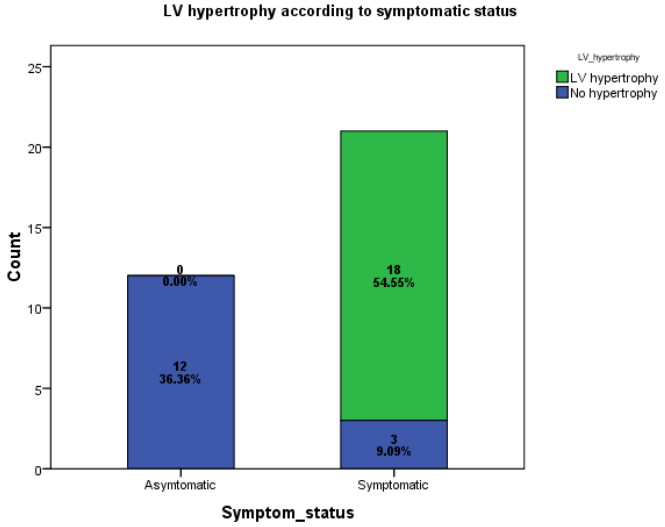
1) LV mass:

The mean LV mass indexed to BSA was 129.76 ± 51.16 gm/m2 in patients and 64.84 ± 15.72 gm/m2 in controls. The distribution was positively skewed with a median of 135.86 gm/m2 and a range of 56.31 to 274.55 gm/m2. Seventeen of the 27 patients with severe AS had LV hypertrophy as defined by ASE criterion of > 95gm/m2 in females and >115 gm/m2 in males. All patients had concentric LV hypertrophy with relative wall thickness more than 0.42.Grouping severe AS patients into mild (96-108 gm/m2 in females and 116-131 gm/m2 in males), moderate (109-121 gm/m2 in females and 132-148 gm/m2 in males) and severe LV hypertrophy (≥ 122 gm/m2 in females and ≥ 149 gm/m2 in males) showed 5 and 12 patients with moderate and severe LV hypertrophy, respectively. None of the patients had mild hypertrophy. Only one patient with moderate AS had severe LV hypertrophy.

When presence of LV hypertrophy was analyzed according to symptomatic status the most important difference was that only 20% of patients without hypertrophy were symptomatic

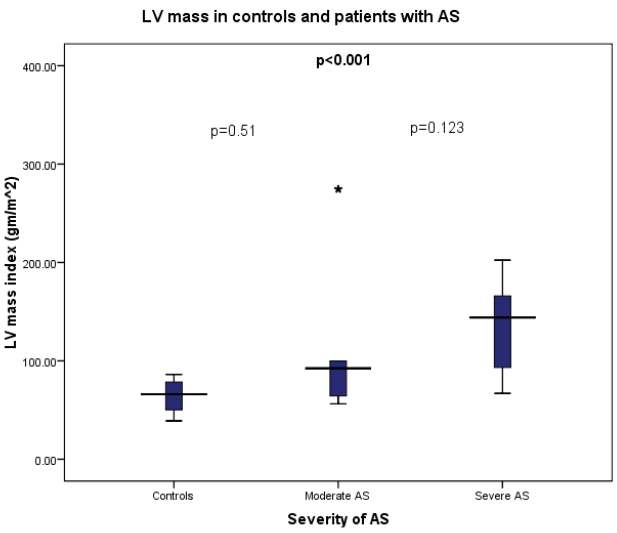
compared to all (100%) of patients with hypertrophy (p<0.001).

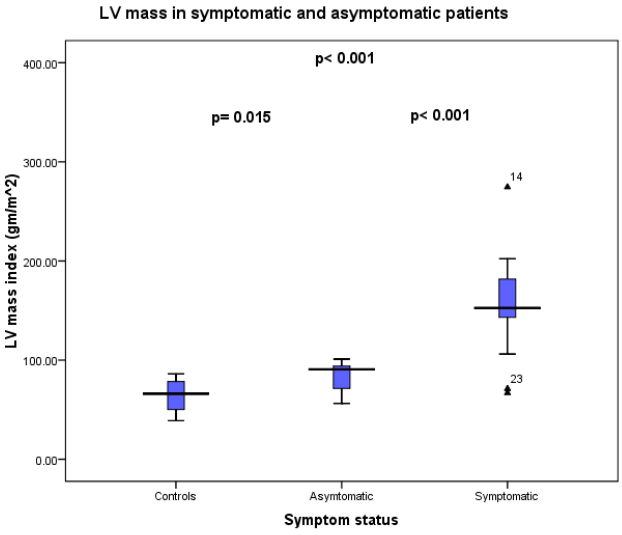




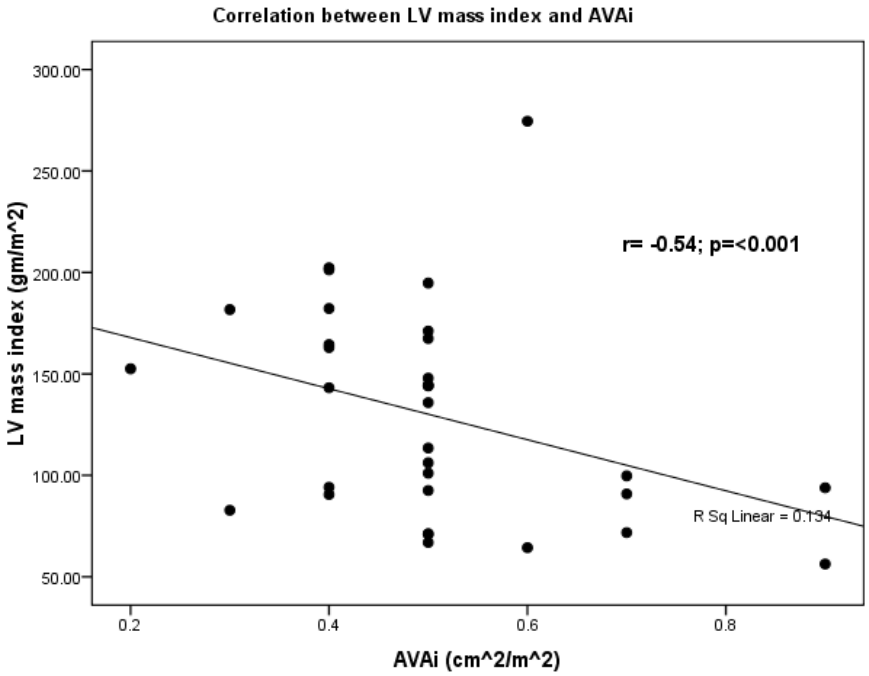
LV mass index was significantly higher in severe AS group compared to controls (Median - 144.1 vs 66.11 gm/m2; p<0.001) but there was no statistical difference between moderate AS and control groups (median 144.1 vs 92.36; p=0.123).

LV mass index was significantly higher in patients with symptomatic AS compared to



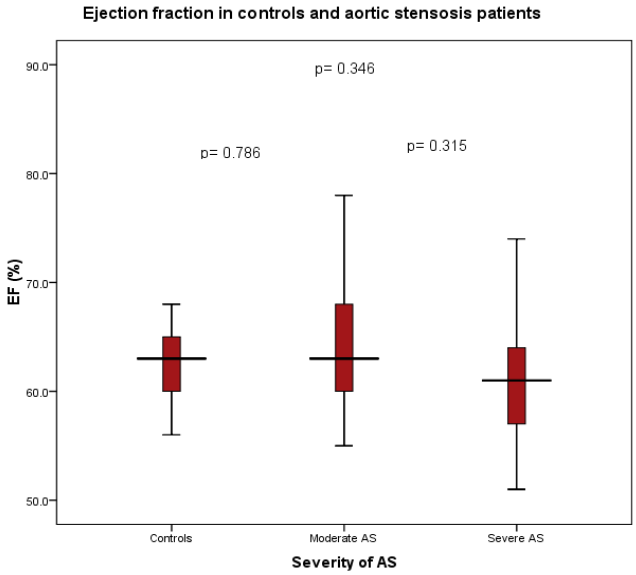


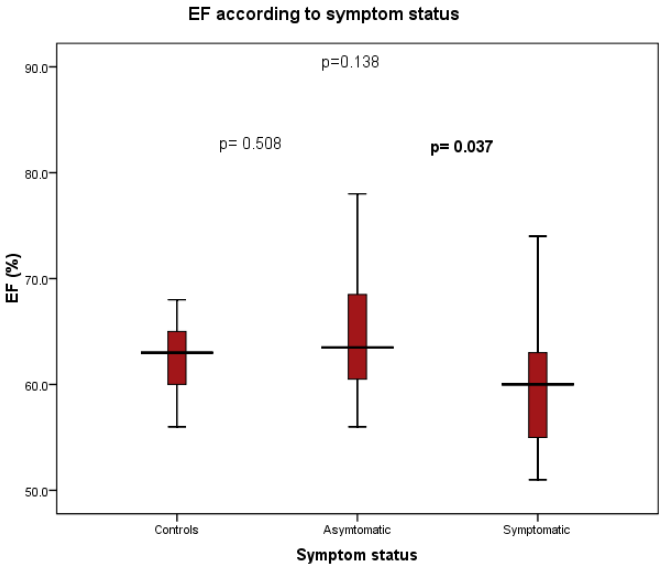
asymptomatic AS (Median -152.55 vs 92.70 gm/m2; p< 0.001).But the correlation between LV mass index and AVA index was only moderate (r=-0.54, p<0.001).



2) Ejection fraction:

The mean ejection fraction (EF) in patients was 61.6 ± 6.2 % and in controls it was 62.4 ± 3.7 %. There was no statistically significant difference between controls and patients with moderate or severe AS. EF was slightly lower in symptomatic patients as compared to asymptomatic patients (59.95 vs 64.50; p=0.037).

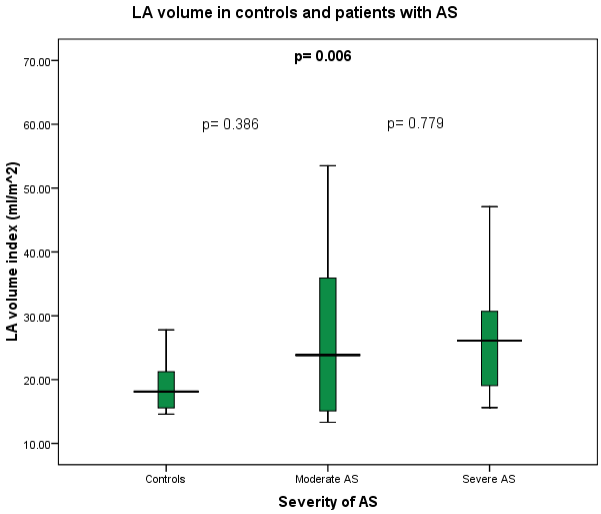


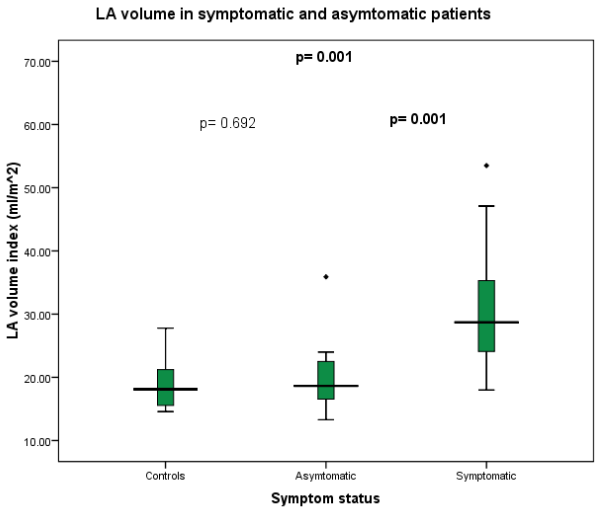


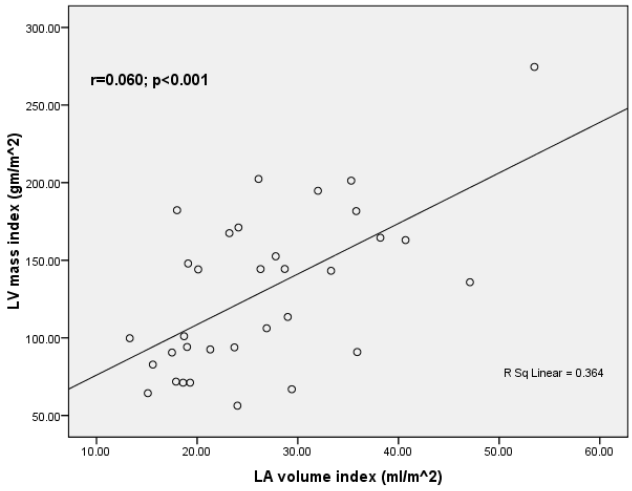
3) LA volume

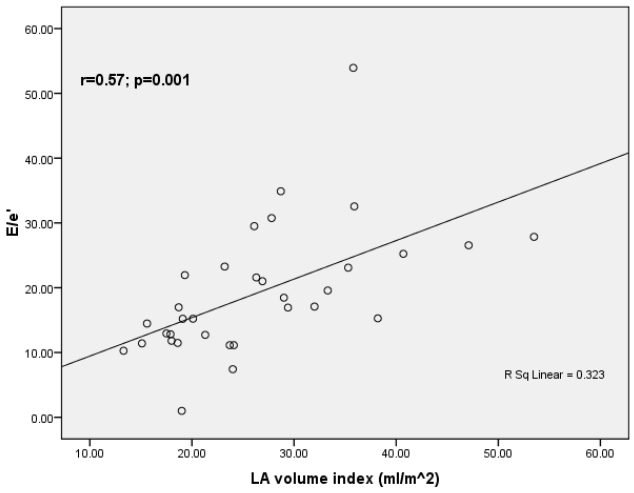
The mean LA volume indexed to body surface area was 26.5 ± 9.48 ml/m^2 in AS patients and 19.26 ± 4.68 ml/m^2 in controls. The distribution of LA volume index was also positively skewed with a median of 24.1 ml/m^2 and a range of 13.30 to 53.50 ml/m^2. Thirteen (39%) patients with AS had LA enlargement as defined by ASE criterion of > 28ml/m^2.Six patients had mild (29-33ml/$m^{2)}$, 4 patients had moderate (34-39ml/$m^{2)}$ and 3 patients had severe LA enlargement (≥ 40ml/m^2). LA volume was significantly higher in patients compared to controls (Median- 24.1 vs 18.1 ml/m^2; p=0.036) but there was no difference between moderate and severe AS groups. There was also no difference in LA volumes between controls and asymptomatic patients (Median 18.1 Vs 18.6; p= 0.692).LA volume was also higher among symptomatic patients

compared to asymptomatic patients (Median - 28.7 vs 18.6 ml/m^2 ; p<0.001).LA volume index correlated moderately with LV mass index (r=0.60 ; p<0.001) and E/e' (r=0.57;p=0.001).







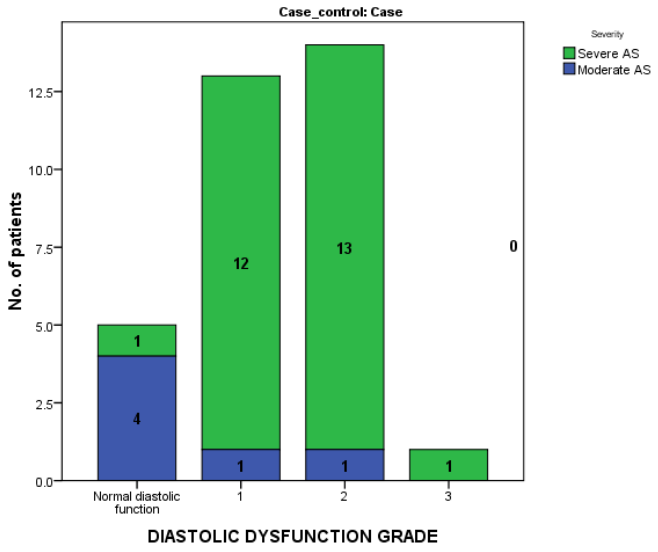


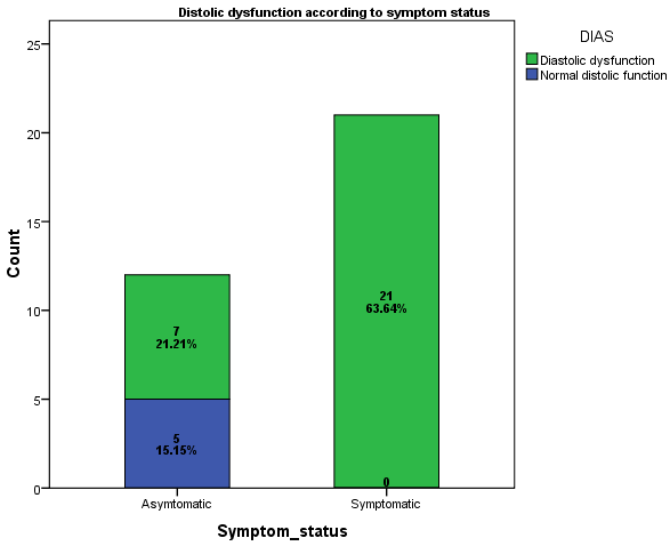
4) LV diastolic function:

The mean ratio of early and late mitral inflow velocities (E/A) was 0.92 ± 0.32 in patients and 1.35 ± 0.34 in controls. The mean deceleration time (DT), medial annular velocity (e') and ratio of early inflow velocity to medial annular velocity (E/e') were 209.58 ± 74.80 msec, 0.05 ±

0.02 and 19.26 ± 9.91 in the patient group, respectively. Corresponding values in controls were 186 ± 20.67, 0.10 ± 0.02 and 8.78 ± 2.07.

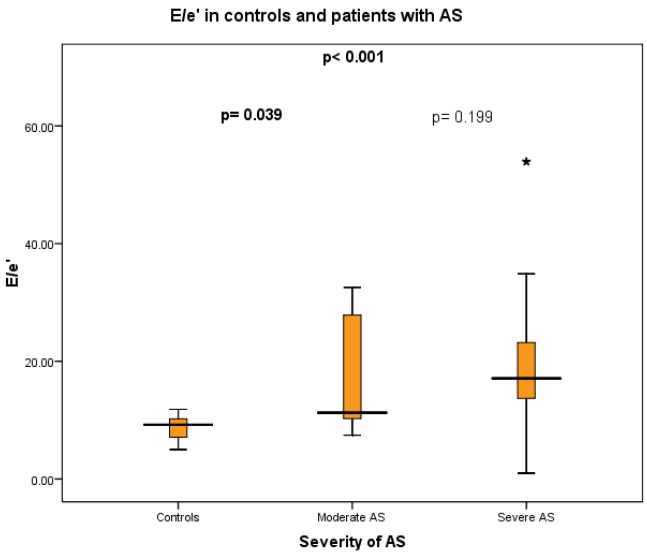
Twenty eight (85%) of patients had some degree of diastolic dysfunction. Among 6 patients with moderate AS, 4 had normal diastolic function, and 1 each had grade I and II diastolic dysfunction. Almost all patients with severe AS had diastolic dysfunction. Among 27 patient with severe AS 12 patients had grade I, 13 patients had grade II and 1 patient had grade III diastolic dysfunction. Among controls, 2 patients over 50 years had grade I diastolic dysfunction.

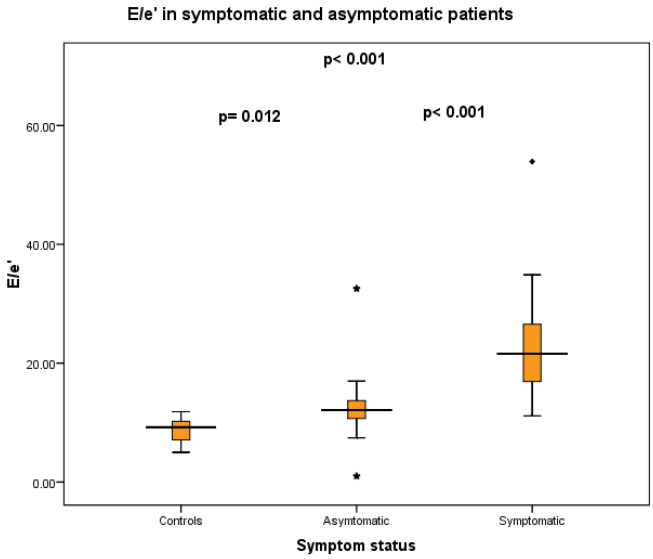




All symptomatic patients had diastolic dysfunction (8 had grade diastolic dysfunction and 13 had grade II or more).In contrast only 7 (58%) of asymptomatic patients (5 grade I and 2 grade II) had diastolic dysfunction .The proportion of patients with LV hypertrophy between symptomatic and asymptomatic patients was statistically significant (p =0.003).

E/e', which is an indirect indicator of LV filling pressure was significantly higher in patients with AS compared to controls (Median -16.98 vs 9.22; p < 0.001).There was no statistically significant difference between moderate and severe AS groups (Median - 11.27 vs 17.09; p=0.199).E/e' was significantly higher in symptomatic patients compared to asymptomatic patients (Median- 21.58 vs 12.10; p<0.001).





5) LV strain

Global longitudinal strain (GLS), global circumferential strain (GCS), global area strain (GAS) and global radial strain were measured. From the longitudinal strain of individual segments basal and apical average segmental strain was calculated. The distribution of echocardiographic variables among symptomatic and asymptomatic and asymptomatic patients is given in Table.3. The mean ± SD and median values of strain are given in the Table 4. Distribution of various strain values in patients with AS is shown in the histograms.

Table.3 Distribution of echocardiographic variables according to symptoms

	Asymptomatic patients (n=12)	Symptomatic patients (n=27)	P value *
Age (yrs)	46.08 ± 12.35	57.19 ± 12.99	**0.03**
Sex			
Male (n)	8	17	ns
Female (n)	4	4	ns
BSA (m^2)	1.72 ± 0.18	1.70 ± 0.19	ns
Systolic BP (mmHg)	122.08 ± 17.82	129.24 ± 21.79	ns
Diastolic BP (mmHg)	73.67 ± 9.23	75.62 ± 9.18	ns
Heart rate (bpm)	78.3 ± 12.8	77.2 ± 14.1	ns
Creatinine clearance (ml/min)	93.82 ± 19.93	80.16 ± 18.54	ns
Hypertension (n)	2	8	ns
Diabetes mellitus (n)	2	5	ns
Smoking (n)	4	8	ns
IVST (cm)	1.22 ± 0.23	1.61 ± 0.25	**<0.001**
PWT (cm)	1.17 ± 0.24	1.59 ± 0.27	**<0.001**
LV EDD (cm)	3.85 ± 0.37	4.32 ± 0.61	**0.001**
LV ESD (cm)	2.36 ± 0.40	2.98 ± 0.51	**0.001**
LV EDV (ml)	73.33 ± 21.75	97.86 ± 35.56	**0.008**
LV ESV (ml)	25.67 ± 7.39	39.91 ± 17.39	**0.008**
LV EF (%)	64.50 ± 5.95	59.95 ± 5.80	**0.037**
LA volume index (ml/m^2)	20.05 ± 5.94	30.19 ± 9.23	**0.001**
LV mass index (ml/m^2)	84.10 ± 14.68	155.86 ± 45.86	**<0.001**
LVOT diameter (cm)	1.99 ± 0.14	2.11 ± 0.35	ns

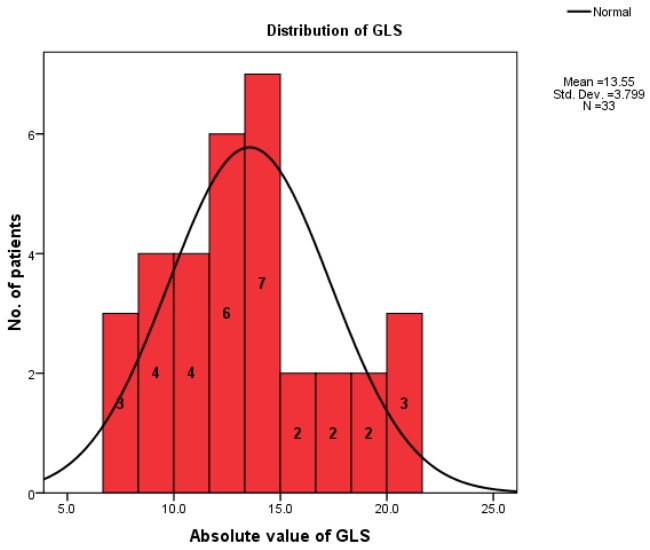
values are mean ±standard deviation . * ns – not significant

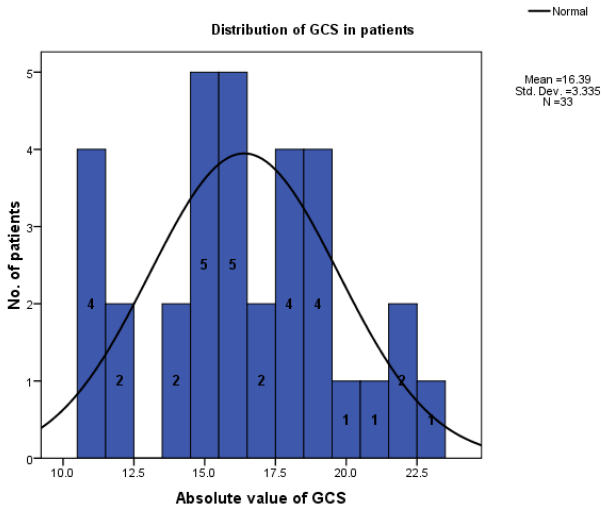
Table 3 continuation

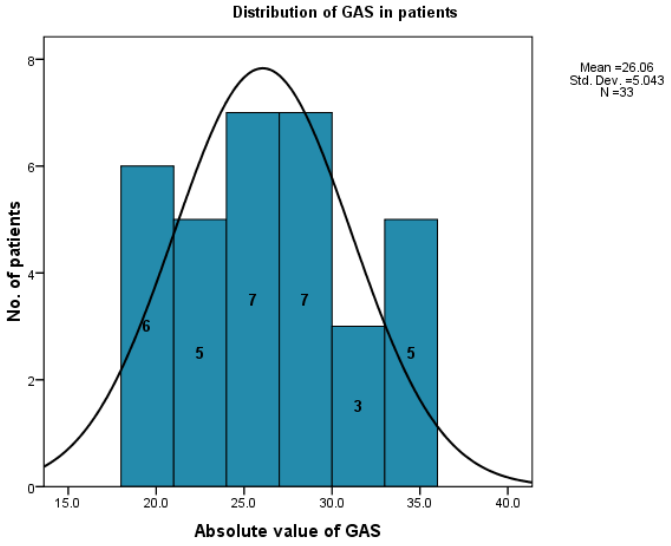
	Aymptomatic patients (n=12)	Symptomatic patients (n=27)	P value *
Mitral valve			
E/A	0.97 ± 0.26	0.88 ± 0.35	ns
Deceleration time (msec)	206.58 ± 96.29	211.29 ± 61.99	ns
Medial e'	0.06 ± 0.02	0.04 ± 0.01	**<0.001**
E/e'	12.93 ± 7.33	22.87 ± 9.49	**<0.001**
AV Vmax (m/sec)	3.87 ± 0.52	4.60 ± 0.67	**0.001**
AV mean gradient (mmHg)	37.02 ± 10.58	56.92 ± 18.15	**0.001**
AV VTI (cm)	80.36 ± 8.78	108.18 ± 25.02	**<0.001**
LVOT VTI (cm)	25.10 ± 4.84	23.04 ± 5.05	ns
AVA index (cm^2/m^2)	0.6 ± 0.2	0.4 ± 0.1	**0.048**

values are mean ±standard deviation . * ns – not significant

Distribution of various strain values in patients with AS







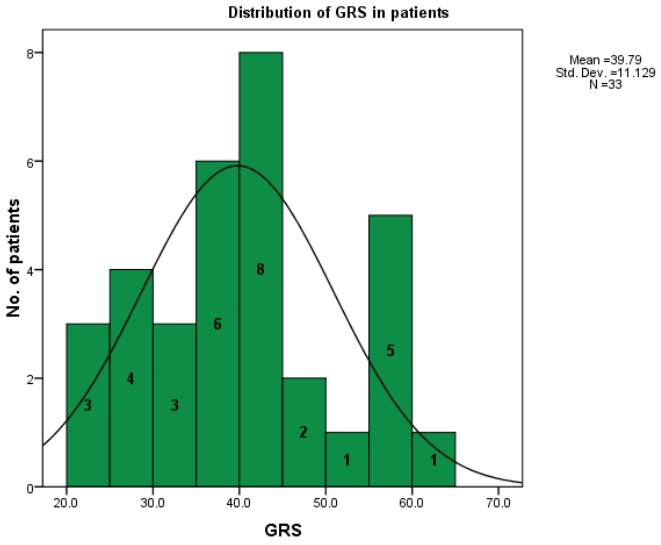


Table 4.Distribution of strain in patients and controls

	Total patients (n=33)	Moderate AS (n=6)	Severe AS (n=27)	Controls (n=10)
Global longitudinal strain (GLS)	-13.56 ± 3.80	-17.57 ± 4.63	-12.66 ± 3.02	-20.77 ± 1.49
Global circumferential strain (GCS)	-16.39 ± 3.34	-18.33 ± 2.33	-15.96 ± 3.40	-16.80 ± 2.82
Global area strain (GAS)	-26.06 ± 5.04	-31.16 ± 4.31	-24.93 ± 4.51	-30.40 ± 3.60
Global radial strain (GRS)	39.70 ± 11.12	49.67 ± 10.39	37.59 ± 10.21	48.9 ± 8.54

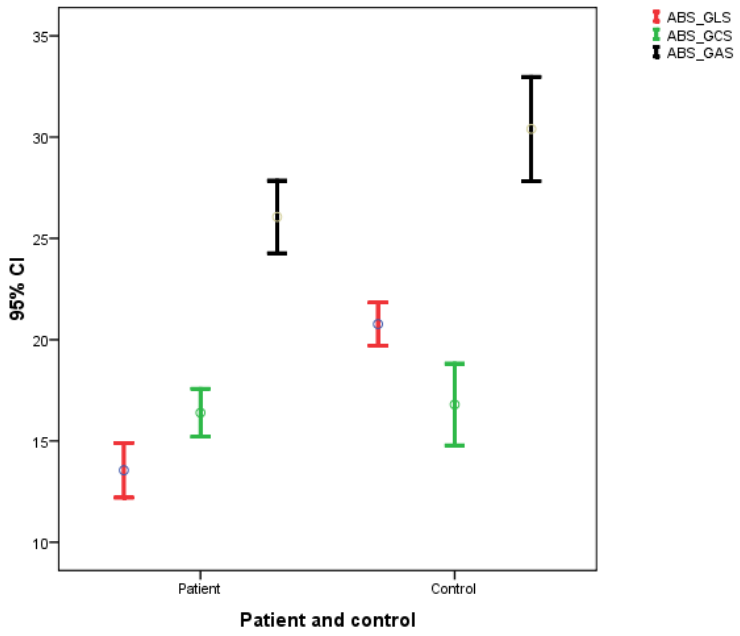
* Values are expressed as mean ± standard deviation and median (25^{th} percentile to 75^{th} percentile)

Table.4 continuation

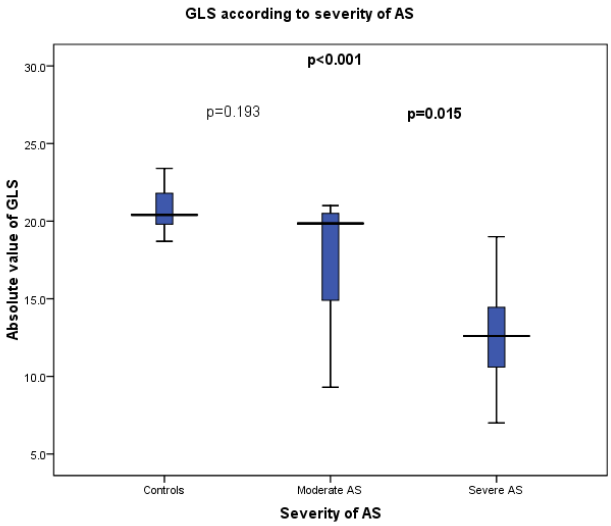
	Total patients (n=33)	Moderate AS (n=6)	Severe AS (n=27)	Controls (n=10)
Basal longitudinal strain	-9.51 ± 4.28	-14.35 ± 4.82	-8.44 ± 3.39	-19.02 ± 1.22
Apical longitudinal strain	-16.82 ± 5.49	-19.37 ± 6.90	-16.2 ± 5.11	-23.38 ± 3.98
NT-ProBNP (pg/ml)^	**453.40** *(141.50 – 1761.00)*	**118.90** *(48.06 – 1669.00)*	**614.20** *(187.70 - 2141.00)*	**34.63** *(20.08 - 72.17)*

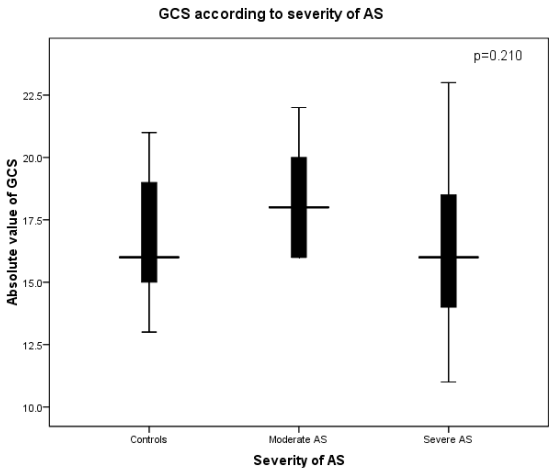
* Values are expressed as mean ± standard deviation and median (25^{th} percentile to 75^{th} percentile)

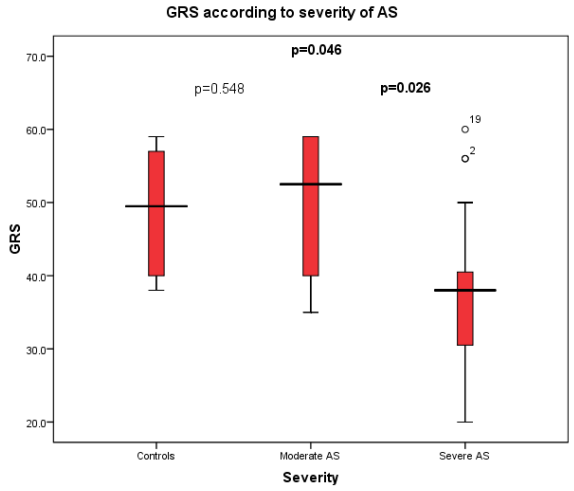
Median GLS, GAS and GRS were significantly lower in patients compared to controls (-13.0 vs -20.4, -26.0 vs -31.5 and 40.0 vs 49.5, respectively; p<0.001, p=0.02 and p=0.018, respectively).Median GCS did not statistically differ between both groups (-16.0 vs -16.0, p= 0.685).

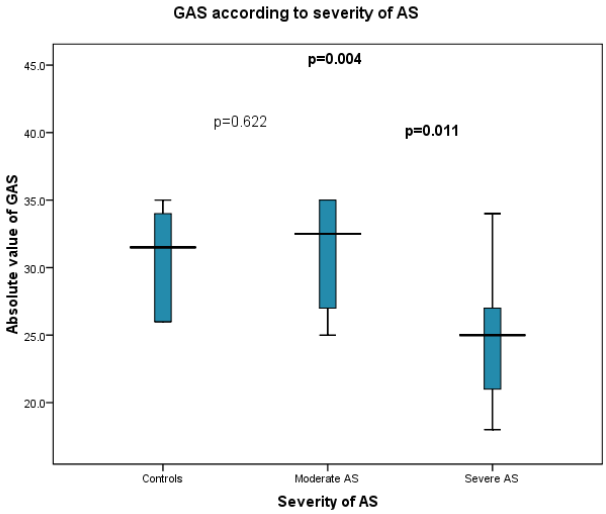


Comparison between moderate and severe AS groups showed significantly lower median GLS,GAS and GRS in patients with severe AS compared to patients with moderate AS (-12.6 vs -19.85 ,-25.0 vs -32.5 , 38.0 vs 52.5 ,respectively; p=0.015,p=0.011,p=0.026,respectively).There was no difference in GCS between severe and moderate AS patients (-16.0 vs -18.0 ; p= 0.095).

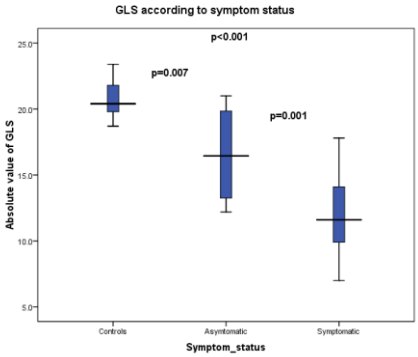


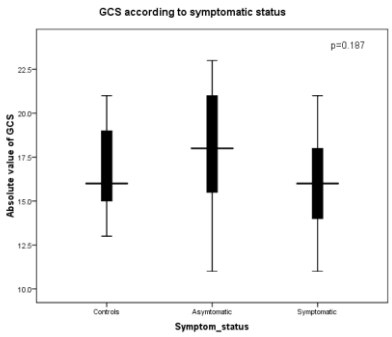


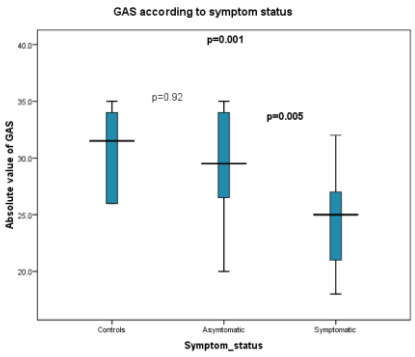


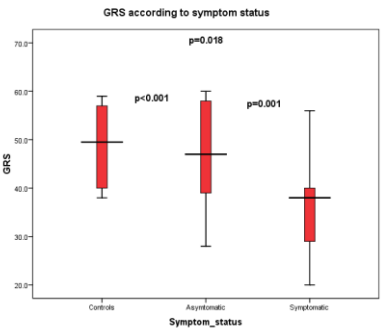


Among symptomatic patients median GLS, GAS and GRS were significantly lower compared to asymptomatic patients (-11.6 vs -16.4, -25.0 vs -29.5, 38.0 vs 47.0, respectively; p= 0.001, p=0.005,p=0.10,respectively.Again,GCS did not differ significantly (-16.0 vs -17.83 ; p=0.087).

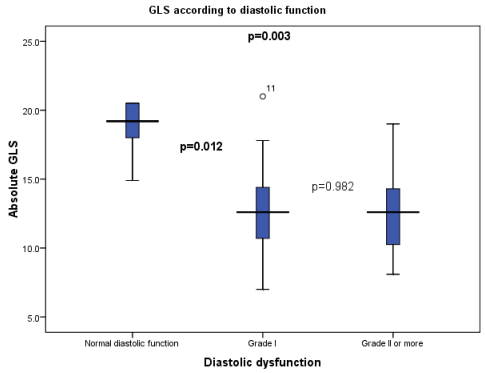


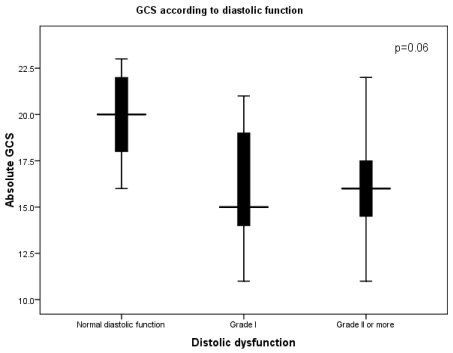


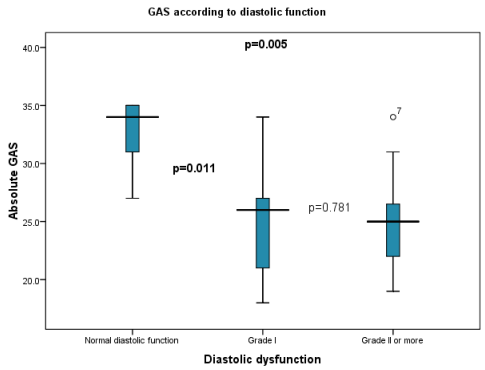


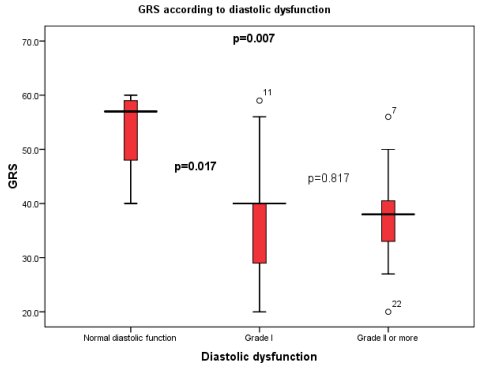


GLS, GAS and GRS were also lower in patients with diastolic dysfunction compared to patients with normal diastolic function (median- -12.6 vs -19.2, -25.0 vs -34.0, 38.0 vs 57.0; p=0.012, 0.014 and 0.021, respectively).But GCS did not differ significantly in patients with diastolic dysfunction (Median- -16.0 vs -20.0; p=0.06).When analyzed for difference in strains between different degrees of diastolic dysfunction there was no significant difference (see figures with p values).









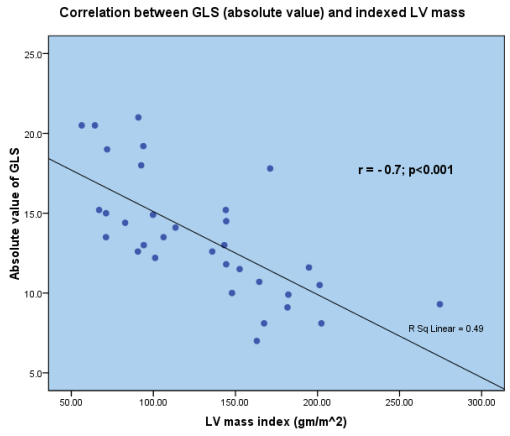
Among patients, when global strain values were correlated with ejection fraction, LA volume index, LV mass index and E/e' only GLS showed strong correlation with LV mass

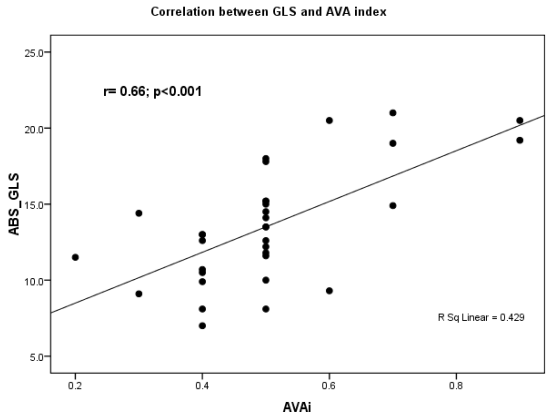
index (Table .5). GCS, GAS and GRS did not show any correlation or only weak correlation .GLS showed modest negative correlation with AVAi (r= -0.66; p<0.001).

Table. 5 Correlation between multidirectional strain and echocardiographic variables

	Absolute GLS	Absolute GCS	Absolute GAS	GRS	NT-ProBNP
Ejection fraction	r= 0.43	**r= 0.53 p=0.001**	r= 0.47	**r= 0.52 p=002**	r= -0.11
LA volume index	r= - 0.35	r= -0.23	r= -0.29	r= -0.25	**r= 0.74 p<0.001**
LV mass index	**r= -0.70 p<0.001**	r= - 0.26	r= -0.39	r= -0.41	**r= 0.68 p<0.001**
E/e'	r= -0.39	r= -0.29	r= -0.34	r= -0.31	**r= 0.56 p=0.001**

Absolute value of global longitudinal strain (GLS) showed strong negative correlation with LV mass index (r= - 0.70; p<0.001) i.e. as LV mass increased absolute GLS (ignoring the negative sign) decreased. Absolute value of GLS showed modest positive correlation with aortic valve area (r= 0.66; p<0.001) i.e. as AVA decreased absolute value of GLS decreased.

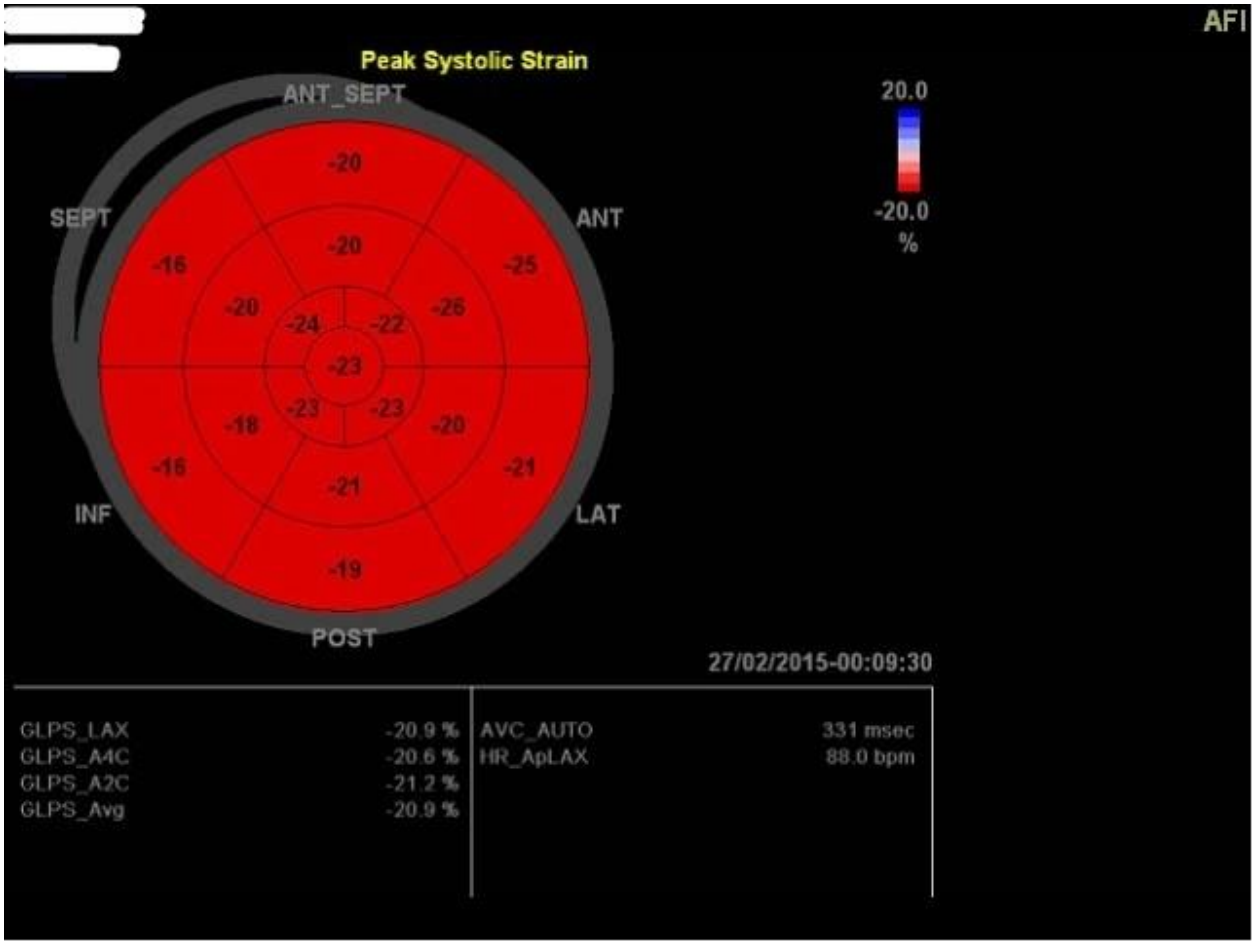




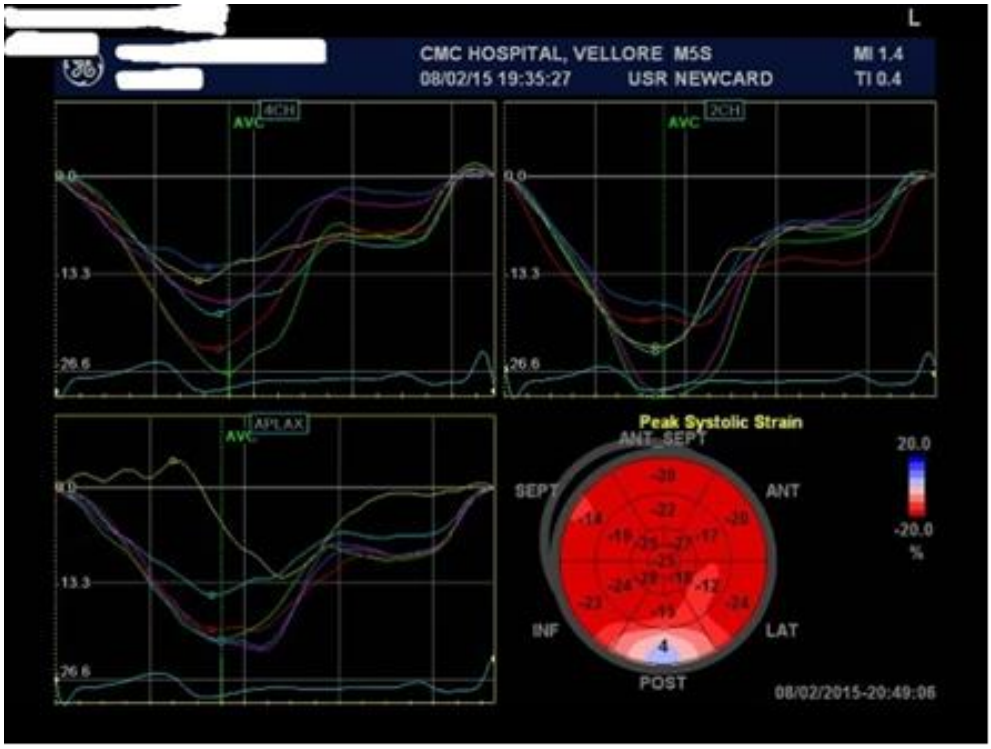
Pattern of longitudinal strain involvement

Visual analysis of bull's eye diagram of longitudinal strain showed two patterns of reduced strain in various segments of LV myocardium.

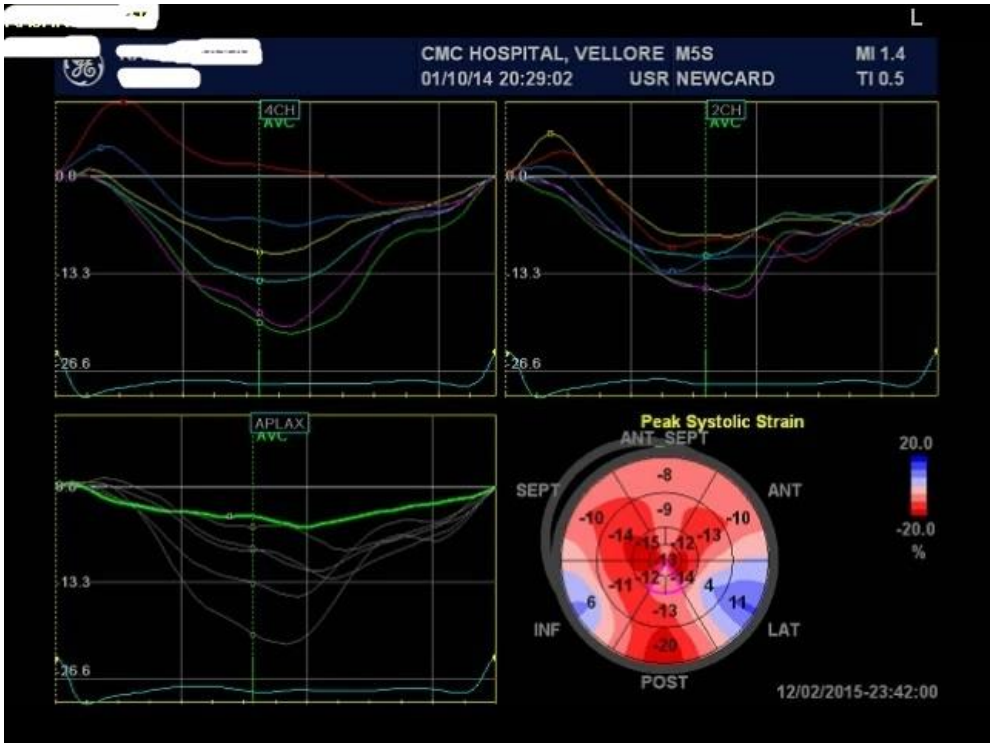
One common pattern was reduced strain in the basal segments, especially the basal anterolateral and inferolateral segments, with preserved strain in mid and apical segments .The second is more extensive reduction of strain.



Longitudinal strain in healthy subject



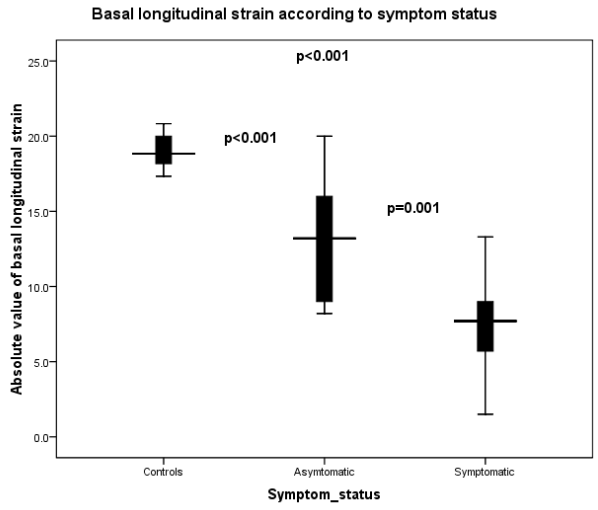
Reduced longitudinal strain in basal segments

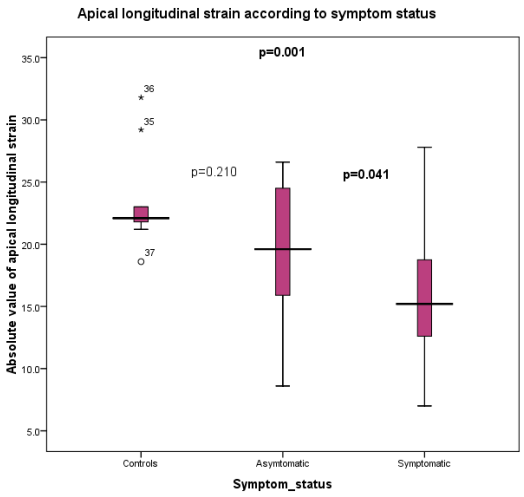


More extensive reduction in longitudinal strain

Although subjective, the first pattern was seen in 8 of the asymptomatic patients. All of the symptomatic patients had the second pattern with more extensive reduction of longitudinal strain. Two asymptomatic patients, both with moderate AS, had near normal strain pattern. None of the symptomatic patients had normal strain pattern.

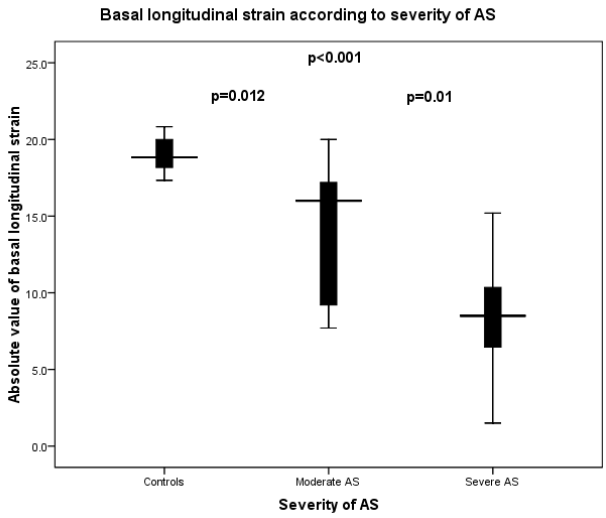
Quantitative analysis of pattern of involvement was done by calculating basal and apical average segmental strain from the longitudinal strain of individual segments. Compared to controls basal longitudinal strain was significantly lower in moderate and severe AS patients (median -18.8 vs -16.0 vs -8.5; p<0.001).But apical longitudinal strain was not significantly different between controls and moderate AS (median -22.1 vs -21.3; p=0.514) and between moderate AS and severe AS (Median -21.3 vs -16.2; p=0.161). When symptomatic and asymptomatic sub-groups were analyzed there was significantly lower basal longitudinal strain in asymptomatic patients compared to controls (median -16.3 vs -18.8; p<0.001) and lower in asymptomatic patients compared to symptomatic patients (median -7.7 vs -13.2; p=0.001).But apical longitudinal strain did not differ between controls and asymptomatic patients





(median -22.1 vs -19.6; p=0.210) . Apical strain was lower in symptomatic compared to symptomatic patients (-15.2 vs -19.6; p=0.41).

When analyzed between controls and sub-groups with moderate and severe AS there was no difference in apical longitudinal strain (Control -22.1 vs moderate AS -21.3; p=0.512 and moderate AS -21.2 vs severe AS -16.2; p=0.161) but basal longitudinal strain was significantly lower in moderate AS patients compared to controls (-16.0 vs -18.8; p=0.012) and lower in severe AS patients vs moderate AS (-8.5 vs -16.0; p=0.01)



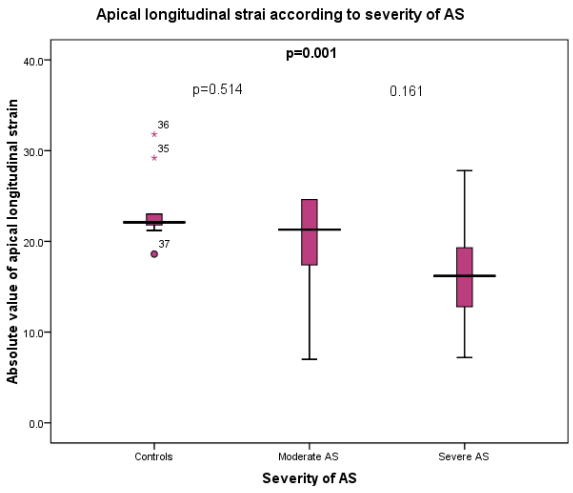


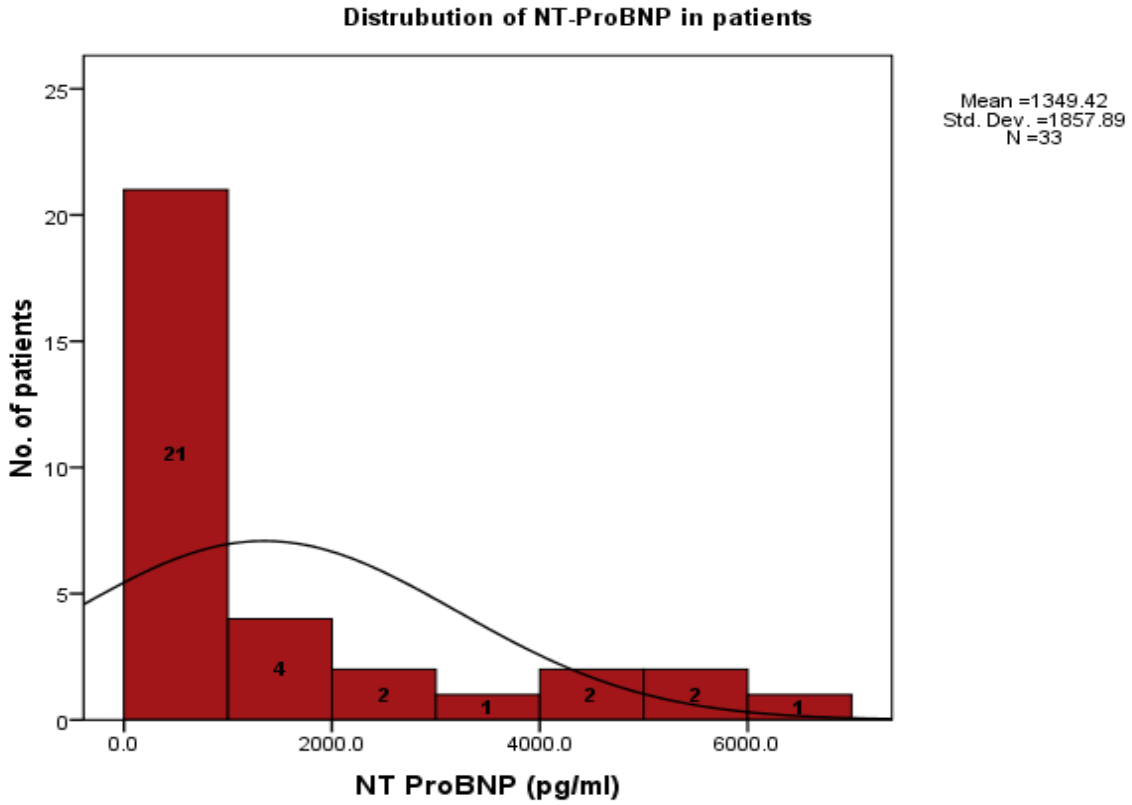
Table 6. Strain and NT-ProBNP values in symptomatic and asymptomatic patients

	Asymptomatic patients (n=12)	Symptomatic patients (n=21)	P value
Global longitudinal strain (GLS)	-16.57 ± 3.44	-11.8 ± 2.84	**0.001**
Global circumferential strain (GCS)	-17.83 ± 3.61	-15.57 ± 2.94	0.087
Global area strain (GAS)	-29.42 ± 5.20	-24.14 ± 3.90	**0.005**
Global radial strain (GRS)	46.92 ± 11.30	35.71 ± 8.95	**0.010**
Basal longitudinal strain	-12.98 ± 3.99	-7.53 ± 3.05	**0.001**
Apical longitudinal strain	-19.43 ± 5.55	-15.32 ± 4.99	**0.041**
NT-ProBNP (pg/ml)	118.90 (73.40 to 174.38)	1191.00 (494.30 to 3844.00)	**<0.001**

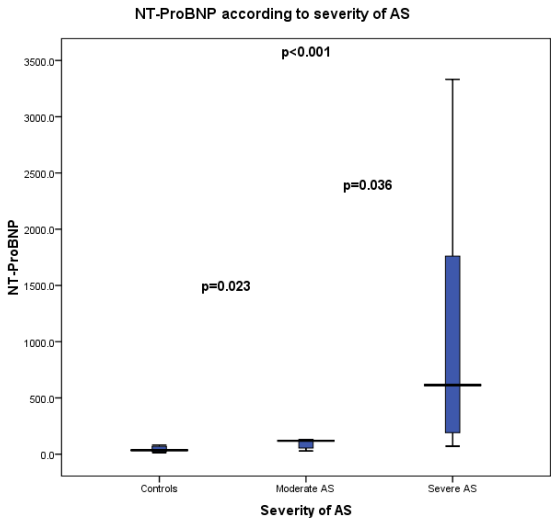
* Values are expressed as mean ± standard deviation and median (25th percentile to 75th percentile)

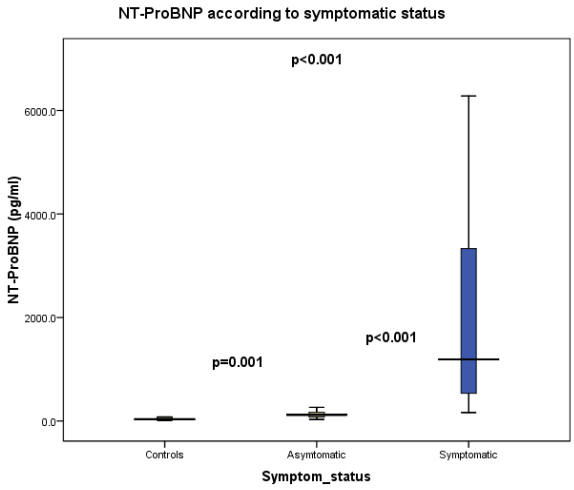
6) NT-ProBNP

Distribution of NT-ProBNP was positively skewed (see figure below).So log transformed NT-ProBNP was used when appropriate. The median NT-ProBNP was significantly higher in patients compared to controls (453.40 vs 34.63 pg/ml; p<0.001).Median NT-pro BNP was significantly higher in severe AS patients compared to moderate AS patients (614.20 vs 118.90 pg/ml p=0.036).

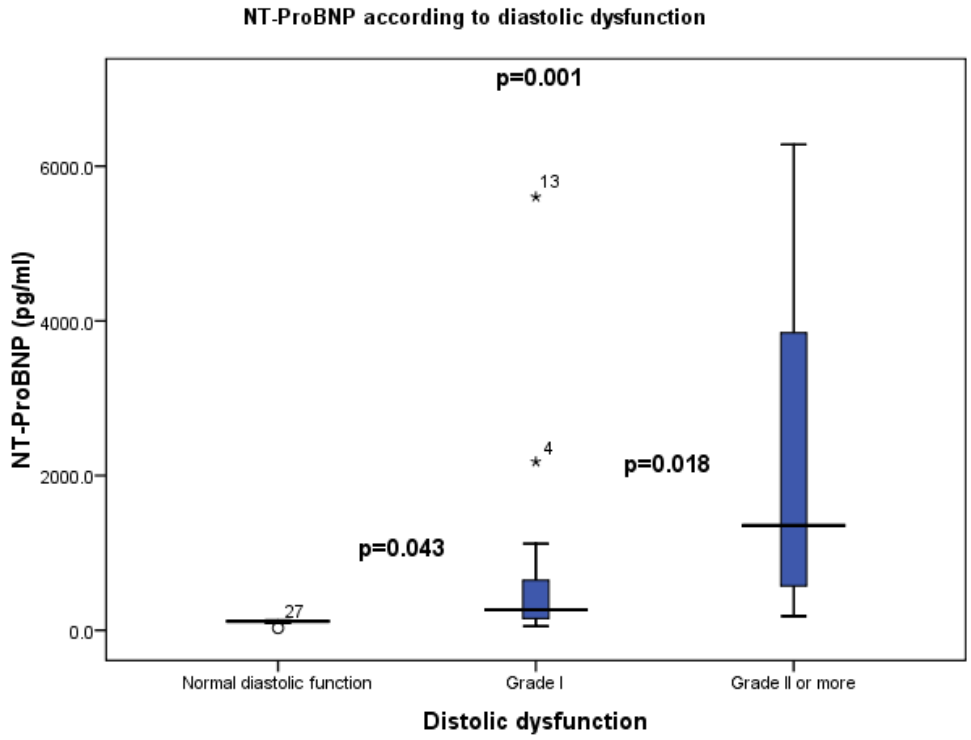


Compared to asymptomatic patients median NT-Pro BNP was higher in patients with symptoms (123.95 vs 204.97; p<0.001).Plasma NT-ProBNP was significantly higher in patients

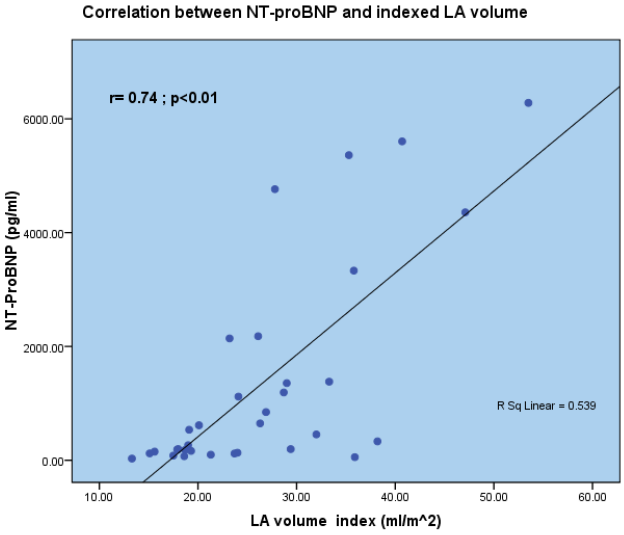


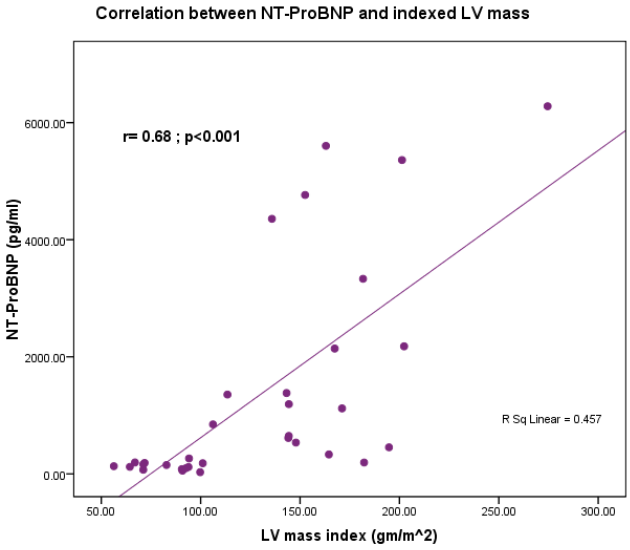


with diastolic dysfunction as compared to patients with no diastolic dysfunction (median- 630.95 vs 117.40 pg/ml; p= 0.001).NT-proBNP also increased with higher grades of LV diastolic dysfunction (median - 1355.00 pg/ml in grade II/III vs 264.20 pg/ml in grade I; p=0.018).

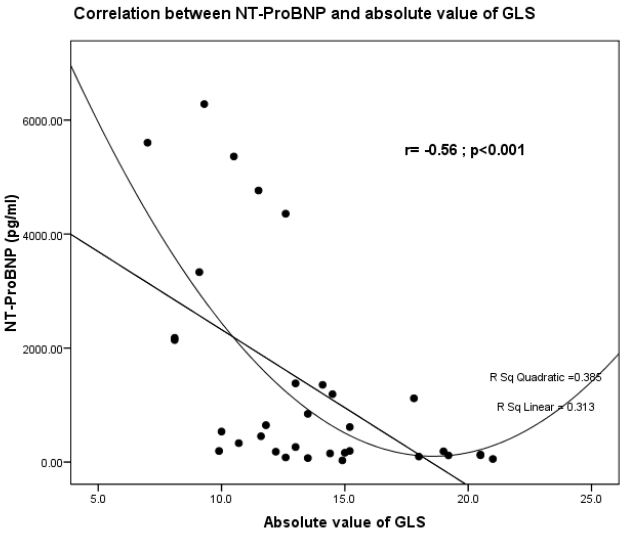


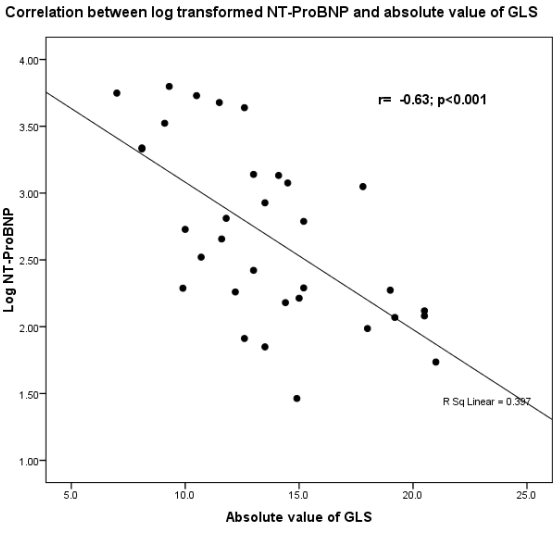
When NT-ProBNP was correlated with other echocardiographic variables there was strong positive correlation with LA volume index (r=0.735;p<0.001). Correlation with LV mass index (r=0.676;p<0.001) and E/e (r=0.564;p=0.001) was modest. There was poor correlation between NT-ProBNP and AVAi (r= -0.38).But log transformation of NT-ProBNP showed correlation of r= - 0.46;p= 0.007.





Scatter plot of absolute GLS and NT-ProBNP showed a modest negative linear correlation but suggested a stronger exponential relationship. Log transformed NT-ProBNP was analyzed and showed better negative correlation with absolute value of GLS (r= -0.63; p<0.001).

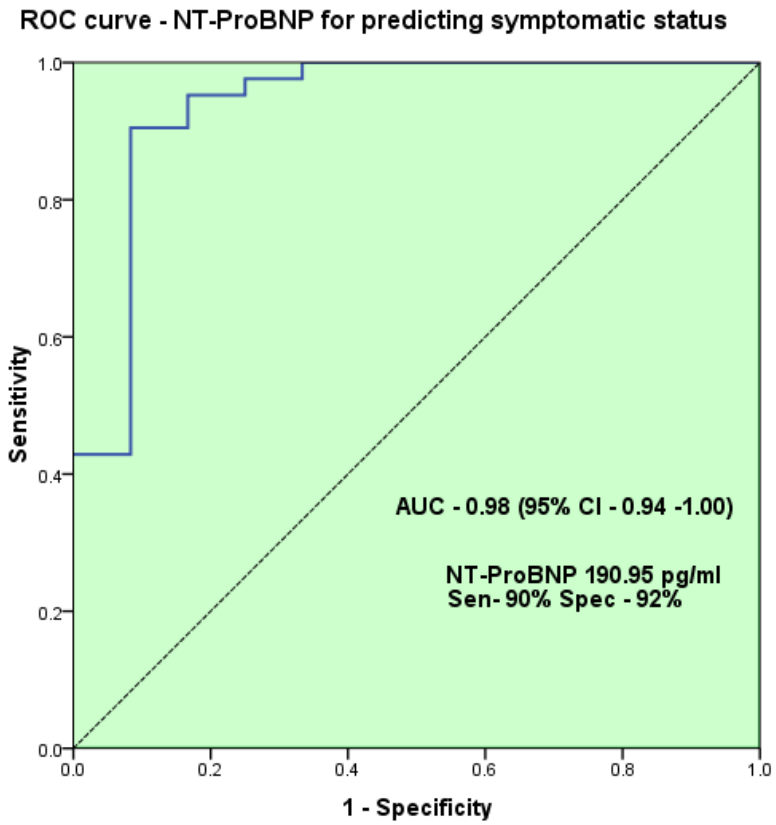




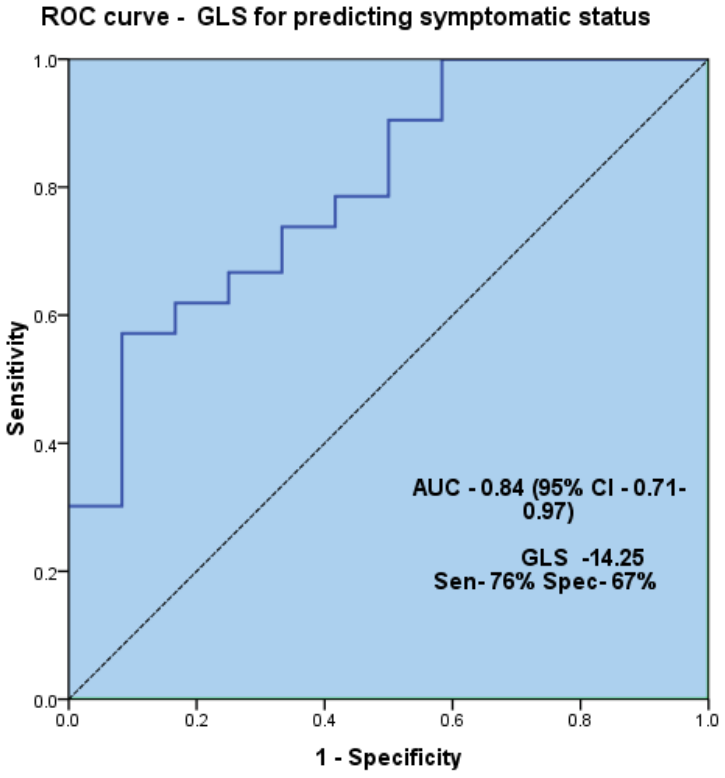
Receiver operator characteristic (ROC) curves

When LV mass index, LA volume index, AVA index, E/e' , LV strains and plasma NT-ProBNP were analyzed for receiver operator characteristics to predict symptom status NT-ProBNP, LV mass index, E/e' and global longitudinal strain (GLS) had best area under curves (AUCs).

AUC for NT-ProBNP for predicting symptomatic status is 0.98 (95% CI 0.93 -1.00) .The best cutoff value was 190.50 pg/ml with a sensitivity of 90.5% and specificity of 91.7% to predict symptoms. AUC for NT-ProBNP for severe AS is 0.778 (95% CI 0.49 - 1.00).The best cut-off for predicting severe AS is 141.5 pg/ml with a sensitivity of 88.9% and specificity of 83.3%.



AUC for GLS was 0.839 (95% CI -0.71 - 0.97).The best cutoff value was -14.25 with 76% sensitivity and 67% specificity to predict symptom status.



Discussion

We studied echocardiographic parameters including multidirectional myocardial strain and plasma NT-ProBNP in patients with moderate to severe aortic stenosis.

The most common cause of aortic stenosis in this study was bicuspid aortic valve unlike in other larger studies where age-related calcific AS predominated (1,42). This perhaps is due to relatively younger population in this study (Mean age of 53.2 years compared to more than 65 years in most larger studies).As this is not a community based study the proportions do not reflect the relative prevalence of various causes. As with any hospital based study, selection bias might have been introduced because this is a tertiary care hospital and most individuals come for definitive therapy. Younger patients in their active years are ready to undergo surgery and present earlier (or referred earlier) compared to older individuals who present only when the symptoms are severe and very elderly tend to avoid surgery altogether. Moreover, the sample size is small for making any generalizations about the true proportions of each cause. Rheumatic heart disease accounted only for two cases, which is not surprising even with the high prevalence of RHD in India as isolated rheumatic aortic stenosis is rare. Although not analysed in this study, if patients with significant mitral valve disease or aortic incompetence are included, more than half of the cases of moderate to severe AS are rheumatic in etiology (see algorithm). Only 7 patients with severe AS were asymptomatic which can also be explained by the selection bias discussed above.

As with all previous studies LV mass was only moderately correlated with aortic valve area (r=-0.54).When analysed as a continues variable the LV mass index increases as severity of AS increases but as defined by current ASE criteria for LV hypertrophy, there was also no significant difference in presence of LVH among moderate and severe AS patients. First, this lack of correlation with severity of AS can be explained by variability of hypertrophic response in among different individuals. This is because there are other factors in addition to the pressure overload influencing the LV response like age, gender, hypertension and genetic variation in the renin–angiotensin system (33,35,36). In this study 37% of patients with severe AS did not have hypertrophy. In previous studies this number was 10–20% (34,37). Second, the cut-off points of 95gm/m2 and 115gm/m2 in women and men, respectively, probably do not hold good for AS patients. When symptoms were considered, all of the patients with LVH were symptomatic and only one-fifth of the patients without LVH were symptomatic. Larger studies in the past also found that echocardiographic LV mass had no predictive value for events in asymptomatic patients. So, LVH is variably related to severity of AS due to multiple factors responsible for hypertrophy but LVH is usually present in symptomatic patients, probably being one of the causes of symptoms due to higher grades of diastolic dysfunction associated with it.

All patients in this study had EF >50%.There was no significant difference in EF among patients with moderate or severe AS. The EF was slightly lower in symptomatic patients but the values in both groups were within normal range. The difference was small and so the sample size is inadequate to study any cut-off for EF

between symptomatic or asymptomatic patients. In larger studies there was no difference among symptomatic or asymptomatic patients and EF did not predict events among patients with EF>50-55%.Two (6%) patients in this study had features of low-flow, low-gradient severe AS with preserved LV function which is similar to reported values of 5-15%.

LA volume index was significantly higher in symptomatic patients and correlated with LV mass (r=0.60) and E/e' (r=0.57).ROC curve for prediction of symptomatic status showed AUC of 0.865 for LA volume index with best cut-off of 22.25 ml/m2 with a sensitivity of 90.5% and specificity of 83%.LA size is a marker of chronic diastolic dysfunction and as expected increases with increasing LV filling pressure, as shown here. Also, LV hypertrophy with its associated diastolic dysfunction and elevated filling pressures increases the LA size. But LA size did not significantly differ between controls and moderate AS and moderate to severe AS patients, again showing that LA size is not just dependent on the severity of AS. All symptomatic patients with AS had diastolic dysfunction (38% grade I and 62% grade II or more).Diastolic dysfunction is one of the important mechanisms which causes symptoms in AS patients with normal LV function. So the results were as expected.

Global longitudinal strain (GLS),global area strain (GAS) and global radial strain (GRS), but not global circumferential strain (GCS) ,were decreased in patients with AS compared to controls decreased with increasing severity of AS. This is in contrast to the study by Lafitte et.al where only GLS was lower but similar to the results of Delgado et.al. Study by Lafitte et.al. included only asymptomatic patients unlike the second study ,which included both symptomatic and asymptomatic

patients (69,73).In our study symptomatic patients formed the majority. So a reasonable conclusion that can be drawn is that longitudinal strain is first affected and as disease progresses strain in other directions is affected and patient becomes symptomatic. This has been explained pathophysiologically by examining the orientation of myofibres in the myocardium (52,53).LV has subendocardial and subepicardial layers with longitudinally oriented fibres and mid wall with circumferentially oriented fibres. Because of increased LV pressure and ischemia, LV subendocardium is the most vulnerable in patients with AS and gets affected first. So, GLS which is a function of subendocardial fibres, is the first to reduce. The absence of significant difference in GCS compared to controls shows that probably GCS, which is a function of mid wall fibres, is the last to get affected. In fact, in the study by Zito et.al. the GCS was significantly elevated compared to controls probably suggesting a phase when there is compensatory increase in GCS (72).

Even though GLS, GRS and GAS were significantly lower in asymptomatic patients compared to controls and decreased further in symptomatic patients, when ROC curve was analysed only GLS had good AUC of 0.839 to predict symptoms. Best cut-off of -14.25 had sensitivity of 76 % and specificity of 67% to predict symptomatic status.

When regional strain was assessed qualitatively by examining bull's eye plots, most asymptomatic patients (8 of 12 patients) had reduced longitudinal strain only in the basal segments with normal values in apical segments. All symptomatic patients had more extensive reduction of strain. Quantitative analysis also showed similar results, with apical strain not differing significantly between controls and

moderate AS and controls and asymptomatic patients. Apical strain was reduced in severe AS patients and symptomatic patients compared to controls. So, basal strain was affected earlier in the course of disease and as disease progresses apical segments are also involved. This can be explained by experimental models which showed that contraction in LV starts from apex and progresses to base (71).In patients with AS the basal segments end their longitudinal contraction against a closed aortic valve and so are subjected to greater stress. This probably results in earlier involvement of basal strain.

NT-ProBNP levels increased in a step wise fashion from controls as severity of AS increased. Same was observed between controls, asymptomatic and symptomatic patients. NT-ProBNP levels also increased with worsening diastolic function. NT-ProBNP had strong correlation with LA volume index (r=0.735) and modest correlation with LV mass index (r=0.676) and E/e (r=0.564).Association between absolute GLS and NT-ProBNP suggested an exponential relationship and log transformed NT-ProBNP showed negative correlation with absolute value of GLS (r= -0.63).This suggested that till GLS decreased to a point there was small elevation in NT-ProBNP and below that point there is an exponential increase. Observations from experimental models have shown increased synthesis and release of BNP once there is transition from compensated to decompensated state of LV myocardium (92). This transition may not be reliably measured by traditional echocardiographic method but probably GLS can detect the transition. To determine this cut-off point larger sample size is required. On ROC curve analysis NT-ProBNP has the best AUC (0.98) compared to any echocardiographic variable for predicting

symptomatic status. A cut-off value of 190.95 pg/ml has sensitivity of 90.5% and specificity of 91.7% to predict symptomatic status.

Conclusions

So, the following conclusions can be drawn from the study

1. Strain along longitudinal (GLS) and radial (GRS) directions is significantly reduced in patients with aortic stenosis with preserved LV ejection fraction compared to healthy subjects. Global area strain is also similarly reduced.

2. In contrast, strain in circumferential direction (GCS) is not significantly reduced even in severe symptomatic patients with AS.

3. Of all the strains, Global longitudinal strain (GLS) is the most significantly decreased.

4. There is regional difference in decrease in longitudinal strain with basal longitudinal strain affected more than apical longitudinal strain.

5. In asymptomatic patients with AS only basal strain is reduced with preserved apical strain and in symptomatic patients apical longitudinal strain is also reduced.

6. Reduced global longitudinal strain (GLS) predicts symptomatic status in AS patient

7. NT-ProBNP levels increase with increasing severity of AS even in asymptomatic patients

8. NT-ProBNP levels are significantly higher in symptomatic patients compared to asymptomatic patients.

9. Compared to all other echocardiographic variables, NT-ProBNP levels best predict symptomatic status in AS patients.

Long term follow up of this cohort of patients will give greater insight into the outcome and the role of these parameters in predicting the outcome. As event rate in

aortic stenosis patients is low long follow up will be required. Once outcome data is available, analysis based on events and determining cut-offs will be more useful for clinical application of the results.

Limitations

1. Small sample size and even lesser number in sub-groups.

2. Selection bias due the nature of patient recruitment with the usual biases of a nonrandomised population.

3. Echocardiography is observer dependent and is associated with observer expectancy bias. Inter-observer and intra-observer variability was not measured for this analysis.

4. This analysis is a cross sectional observational study and patient outcomes are not known. So translation into clinically meaningful data requires long-term follow.

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Abbreviations

3DE	3-dimensional echocardiography
4D Auto LVQ	4-dimensional automatic left ventricular quantification
A3C	Apical 3-chamber view
A5C	Apical 5-chamber view
AFI	Automated functional imaging
APLAX	Apical long axis view
AS	Aortic stenosis
ASE	American Society of Echocardiography
AV	Aortic valve
AVA	Aortic valve area
AVAi	Aortic valve area index
BNP	Brain natriuretic peptide
CWD	Continuous wave doppler
ECLIA	Electrochemiluminisccence assay
EF	Ejection fraction
ESE	European Society of Echocardiography
GAS	Global area strain
GCS	Global circumferential strain
GLS	Global longitudinal strain
GRS	Global radial strain
LA	Left atrium
LV	Left ventricle
LVEDP	Left ventricular end diastolic pressure
LVH	Left ventricular hypertrophy
LVOT	Left ventricular outflow tract
MAPSE	Mitral annular peak systolic excursion systolic
NT-ProBNP	N-terminal pro-brain natriuretic peptide
ROC	Receiver operator characteristics
ROI	Region of interest
RT3DE	Real-time 3-dimensional echocardiography
STE	Speckle tracking echocardiography
STI	Speckle tracking imaging
VTI	Velocity time integral

Patient consent form

Study Title: **The role of extended Vs conventional echocardiographic parameters and NT-ProBNP in assessing aortic stenosis**.

Participants name:

ID:

Date of birth /Age:

I _____ son /daughter of _____

1. Declare that I have read the information sheet provided to me regarding this study and have clarified doubts that I had about the study.

2. I also understand that my participation in this study is entirely voluntary and that I am free to withdraw permission to continue to participate at any time without affecting my usual treatment or my legal rights.

3. I understand that the study staff and institutional ethics committee members will not need my permission to look at my health records even if I withdraw from the trial. I agree to this access.

4. I understand that my identity will not be revealed in any information released to third parties or published.

5. I voluntarily agree to take part in this study.

Name:

Signature:

Date:

Name of the witness:

Relationship to the participant:

Date:

Space for thumb impression

Data collection sheet

Patient details

Hospital ID: **Study ID:**

Name: Age: Sex :

Address:

Phone 1: Phone 2:

E-Mail:

SYMPTOMS

Dyspnea:

Duration - Progression -

Current Severity - NYHA class:

PND - Orthopnea-

Any other characteristics-

Pre-syncope /syncope:

Number - Duration -

Exertional /rest -

If syncope: Posture at onset- Preceding palpitations-

Duration of LOC- Involuntary movements-

Symptoms after recovery-

Any other characteristics-

Chest pain

Character: Angina / Atypical / Non-cardiac

If angina: Severity - CCS class: Any rest pain -

If yes - episodes/duration Any nocturnal angina-

Any other characteristics-

Palpitations:

Duration - Frequency-

Exertional /rest - Duration of each episode -

Regular or irregular- Any ECG documentation -

Any other characteristics -

Other symptoms:

Other diseases/ Past medical history:

Diabetes:

Systemic hypertension:

Peripheral vascular disease:

Rheumatic fever-

Stroke: Ischemic/Haemorrhagic/lacunar/unknown Site (on imaging)-

Clinical manifestation:

TIA: GI bleeding:

Others:

Smoking: Duration - Number- Pack years-

If reformed- Started - Quit -

Medications:

Physical examination

Height: BSA:

Weight: BMI:

Blood pressure:

Arterial pulse: Rate - Regular/irregular -

Character - R/F delay -

RR:

JVP:

Inspection and palpation:

Chest wall-

Apex-

Parasternal pulsation- Epigastric pulsation- 2nd left space-

Palpable sounds-

Thrill-

Auscultation:

S1- S2-

S3- S4-

Clicks - Murmurs -

ECG:

CXR:

BIOCHEMISTRY:

eGFR:

Plasma NT-ProBNP

Echocardiography

M-Mode and 2D:

IVSd- LVPWd-

LVIDd- LVIDs-

FS: EDV (Teich): ESV (Teich): EF (Teich):

EDV (Simp): ESV (Simp): EF (Simp):

Etiolgy of aortic stenosis :

Annulus - Sinuses- STJ - Asc.Ao -

LVOT - Diameter - Area -

LA diameter - LA volume -

LV mass -

Doppler Measurements & TDI

MV E max vel: MV A max vel:

MV E/A: Decceleration time:

Medial e': Lateral e' : E/e:

Aortic valve peak velocity (max. vel view):

Aortic valve mean gradient(max. gdt view):

AV VTI:

LVOT VTI:

Aortic valve area (continuity equation):

Global longitudinal strain (acquired by 2D): GLS by 4D:

Global circumferential strain (4D): Global area strain (4D):

Global Radial strain (4D)

Raw data spreadsheets

S.No.	I D Number	Age	Sex	Symptom Status	NYHA Class	Syncope	Angina	CCS Class	Diabetes	HTN	Smoking
1	030371G	26	F	Y	2	N	N	-	N	N	N
2	035188G	40	M	Y	2	N	N	-	Y	N	Y
3	039834G	53	M	Y	2	N	N	-	N	Y	N
4	042348G	69	M	Y	3	Y	Y	3	N	Y	N
5	252598C	58	F	N	1	N	N	-	N	N	N
6	048707G	65	M	N	1	N	N	-	Y	Y	Y
7	388640D	63	M	N	1	N	N	-	Y	N	Y
8	052887G	55	M	Y	2	N	N	-	N	Y	Y
9	055340G	32	M	N	-	N	N	-	N	N	Y
10	056485G	65	M	Y	2	N	N	-	Y	N	N
11	976380A	32	M	N	-	N	N	-	N	N	N
12	065458G	36	F	N	1	N	N	-	N	N	N
13	422483D	74	M	Y	2	N	Y	2	N	Y	Y
14	065312G	56	M	Y	2	N	Y	2	Y	N	Y
15	093108G	39	M	N	1	N	N	-	N	Y	N
16	134430G	72	M	Y	2	N	N	-	N	N	N
17	138297G	50	F	N	1	N	N	-	N	N	N
18	136491G	66	M	Y	2	N	N	-	N	N	N
19	142243G	31	M	N	1	N	N	-	N	N	Y
20	141984G	53	M	Y	2	N	Y	2	Y	N	Y
21	047787G	50	F	Y	2	N	N	-	N	N	N
22	146033G	54	M	Y	3	N	N	-	N	N	N
23	146490G	70	M	Y	2	N	N	-	N	N	N
24	149832G	64	F	Y	2	N	N	-	N	N	N
25	855354B	54	M	Y	2	N	N	-	N	N	Y
26	146622G	35	M	Y	2	N	N	-	N	N	N
27	837185C	46	M	N	-	N	N	-	N	N	N
28	147633G	55	M	Y	2	N	N	-	N	Y	Y
29	131663G	56	M	N	-	N	N	-	N	N	N
30	063870G	64	M	Y	2	N	Y	2	N	Y	Y
31	946271F	77	M	Y	2	N	N	-	Y	N	N
32	030215G	45	F	N	-	N	N	-	N	N	N
33	166243G	49	M	Y	2	N	Y	2	Y	Y	N
34	CONTROL 1	51	M	-	-	-	-	-	N	N	N
35	CONTROL 2	26	M	-	-	-	-	-	N	N	N
36	CONTROL 3	65	F	-	-	-	-	-	N	N	N
37	CONTROL 4	38	F	-	-	-	-	-	N	N	N
38	CONTROL 5	29	F	-	-	-	-	-	N	N	N
39	CONTROL 6	29	M	-	-	-	-	-	N	N	N
40	CONTROL 7	55	M	-	-	-	-	-	N	N	N
41	CONTROL 8	44	M	-	-	-	-	-	N	N	N
42	CONTROL 9	55	M	-	-	-	-	-	N	N	N
43	CONTROL 10	74	M	-	-	-	-	-	N	N	N

Raw data spreadsheets

S.No.	Height (Cm)	Weight (Kg)	BSA (m^2)	BMI (kg/m^2)	SBP (mmHg)	DBP (mmHg)	HR (bpm)	Cr. Cl (ml/min)	IVSd (cm)	PWd (cm)	LVEDD (cm)	LVESD (cm)	LVOT diameter (cm)
1	148	53.5	1.46	24.40	94	74	89	102.0	1.4	1.4	3.5	2.4	1.7
2	167	54.0	1.62	19.40	116	66	66	110.0	1.4	1.4	4.0	2.8	2.4
3	160	80.0	1.83	31.20	148	84	74	80.0	1.4	1.5	4.6	3.0	2.1
4	163	78.0	1.84	29.40	116	78	72	71.0	1.6	1.7	5.0	3.5	2.3
5	141	45.0	1.31	22.60	138	84	84	73.0	0.9	0.9	4.1	2.7	1.9
6	176	56.5	1.69	18.24	150	78	62	57.0	1.4	1.2	3.7	2.4	1.8
7	171	73.0	1.84	24.60	120	70	88	94.0	1.0	1.0	4.1	2.5	2.0
8	169	87.4	1.98	30.60	160	98	108	92.0	2.0	1.7	4.4	3.2	2.3
9	170	76.9	1.88	26.61	124	82	106	124.0	1.6	1.6	3.8	2.4	2.2
10	166	69.3	1.77	25.15	124	84	86	84.0	1.6	1.8	3.6	2.4	1.7
11	176	60.3	1.74	19.47	100	58	66	123.0	1.1	0.9	4.6	3.0	2.1
12	151	53.4	1.48	23.42	104	64	83	99.0	1.0	0.9	3.5	2.1	2.2
13	163	78.0	1.84	29.36	142	62	59	36.0	1.9	1.9	4.4	2.8	2.1
14	174	72.6	1.87	23.98	122	80	70	104.0	2.1	2.1	5.4	3.9	2.4
15	174	75.3	1.90	24.87	148	75	69	90.0	1.2	1.2	4.0	1.6	2.0
16	167	60.5	1.68	21.69	142	68	62	86.0	1.5	1.6	4.4	2.9	1.7
17	166	72.5	1.81	26.31	120	72	88	110.0	1.6	1.4	3.5	2.1	1.8
18	154	44.0	1.38	18.55	100	72	66	71.0	1.7	1.5	3.6	2.4	1.7
19	171	61.0	1.71	20.86	94	62	68	99.0	1.4	1.5	3.8	2.1	2.1
20	167	70.0	1.79	25.10	120	82	56	58.0	1.8	1.7	4.3	3.1	1.9
21	150	40.0	1.30	17.78	110	70	57	54.0	1.6	1.4	4.2	2.8	1.9
22	170	71.5	1.83	24.74	98	64	91	86.0	1.6	1.4	5.2	3.8	2.6
23	162	56.5	1.60	21.53	132	86	69	83.0	1.5	1.4	2.9	1.8	1.8
24	148	0.0	1.49	25.75	154	70	88	82.0	1.5	1.6	4.4	3.1	2.0
25	168	55.0	1.62	19.49	160	74	73	73.0	1.6	1.6	4.3	2.8	2.0
26	165	67.0	1.74	24.61	122	82	94	84.0	1.7	1.7	4.4	3.1	3.1
27	174	74.2	1.89	24.51	112	84	79	98.0	1.1	1.1	4.2	2.9	2.0
28	176	78.9	1.95	25.47	110	70	83	100.0	2.0	1.9	4.7	3.3	2.3
29	164	70.5	1.77	26.21	125	85	65	74.0	1.1	1.1	3.3	2.0	1.9
30	156	63.0	1.63	25.90	160	82	86	85.0	1.5	1.4	4.9	3.4	2.1
31	156	55.8	1.54	22.90	124	62	79	51.6	1.2	1.2	4.8	3.5	2.0
32	169	56.9	1.65	19.90	130	70	81	84.9	1.2	1.2	3.6	2.5	1.9
33	160	93.3	1.96	36.40	160	80	93	90.6	1.2	1.4	3.8	2.6	2.2
34	175	76.1	1.92	24.85	132	72	56	109.9	1.1	1.1	4.5	3.3	2.3
35	132	82.0	1.61	47.06	130	74	81	121.0	0.8	0.8	4.9	3.2	2.4
36	174	62.0	1.75	20.48	120	82	68	53.6	0.9	1.0	3.7	2.5	1.9
37	152	64.5	1.61	27.92	130	72	97	67.0	0.7	0.8	4.0	2.7	1.8
38	161	76.0	1.80	29.32	124	78	88	121.7	0.8	0.8	3.2	2.3	2.0
39	174	64.5	1.78	21.30	130	80	86	128.6	0.9	0.9	4.2	2.9	2.0
40	170	102.1	2.12	35.33	112	74	96	83.3	1.2	1.1	3.9	2.7	2.2
41	161	59.8	1.63	23.07	110	70	68	76.3	1.0	0.9	3.6	2.2	1.8
42	161	61.2	1.64	23.61	138	78	58	91.0	1.0	1.0	4.5	3.0	1.9
43	160	60.2	1.62	23.52	136	82	62	50.6	1.2	1.2	3.9	2.7	2.2

Raw data spreadsheets

S.No.	LA volume index (ml/m^2)	LV mass index (gm/m^2)	EDV (ml)	ESV (ml)	EF (%)	E vel (m/sec)	A vel (m/sec)	E/A	Dec. time (msec)	Medial e' (m/sec)	E/e'	Etiology
1	29.0	113.5	56.0	22.0	60	1.31	0.79	1.65	267.00	0.07	18.46	BAV
2	24.1	171.1	101.0	40.0	60	0.57	0.80	0.71	238.00	0.05	11.13	UK
3	19.1	147.9	110.0	44.0	60	0.91	1.00	1.10	247.00	0.06	15.20	BAV
4	26.1	202.4	96.0	42.0	56	0.59	1.07	0.55	260.00	0.02	29.50	ARC
5	23.7	93.9	80.0	32.0	60	0.70	0.81	0.86	134.00	0.07	11.13	BAV
6	19.0	94.1	59.0	22.0	63	0.60	0.81	0.74	290.00	0.05	1.00	ARC
7	17.9	71.8	110.0	34.0	69	0.89	0.72	1.24	170.00	0.07	12.82	ARC
8	38.2	164.5	128.0	59.0	54	0.30	1.26	0.24	70.00	0.02	15.27	UK
9	18.7	101.1	75.0	31.0	59	1.05	0.81	0.81	141.00	0.06	16.98	BAV
10	26.3	144.3	62.0	23.0	64	0.87	1.26	0.69	262.00	0.04	21.58	ARC
11	35.9	90.9	113.0	36.0	68	1.61	1.64	0.98	468.00	0.05	32.55	RHD
12	24.0	56.3	64.0	14.0	78	0.73	0.69	1.06	140.00	0.10	7.43	BAV
13	40.7	163.0	124.0	38.0	69	0.72	1.13	0.64	219.00	0.03	25.23	ARC
14	53.5	274.6	107.0	48.0	55	0.98	0.81	1.21	156.00	0.04	27.85	UK
15	17.5	90.6	63.0	22.0	64	0.68	0.86	0.79	213.00	0.05	12.94	BAV
16	27.8	152.6	83.0	29.0	65	0.94	0.85	1.11	161.00	0.03	30.75	ARC
17	15.6	82.8	72.0	28.0	61	0.55	0.81	0.67	130.00	0.04	14.47	BAV
18	33.3	143.2	59.0	15.0	74	0.81	0.77	1.06	178.00	0.04	19.57	ARC
19	21.3	92.6	68.0	21.0	70	0.82	0.57	1.45	174.00	0.06	12.72	BAV
20	35.3	201.3	72.0	34.0	52	0.78	0.73	1.07	153.00	0.03	23.09	ARC
21	47.1	135.9	53.0	18.0	67	0.88	0.82	1.08	342.00	0.03	26.55	UK
22	23.2	167.4	188.0	87.0	54	0.69	0.82	0.84	195.00	0.03	23.27	UK
23	19.3	71.1	53.0	21.0	61	0.92	1.33	0.69	262.00	0.04	21.94	BAV
24	32.0	194.8	107.0	41.0	61	0.50	1.48	0.34	235.00	0.03	17.09	ARC
25	28.7	144.4	88.0	40.0	55	1.41	1.38	1.02	298.00	0.04	34.88	RHD
26	20.1	144.1	160.0	68.0	58	0.76	0.87	0.88	187.00	0.05	15.21	BAV
27	13.3	99.8	84.0	32.0	62	0.76	0.58	1.33	155.00	0.07	10.27	BAV
28	35.8	181.7	118.0	44.0	63	1.62	1.03	1.58	172.00	0.03	53.93	BAV
29	15.1	64.4	56.0	20.0	64	0.77	0.74	1.05	217.00	0.07	11.41	BAV
30	18.0	182.2	124.0	52.0	58	0.59	1.02	0.58	195.00	0.05	11.81	ARC
31	26.9	106.2	95.0	46.0	51	0.70	1.06	0.67	132.00	0.03	21.00	ARC
32	18.6	71.1	36.0	16.0	56	0.50	0.76	0.67	247.00	0.04	11.48	BAV
33	29.4	66.9	71.0	27.0	62	0.98	1.19	0.83	208.00	0.06	16.93	UK
34	14.6	76.9	100.0	40.0	60	0.78	0.48	1.65	178.00	0.11	6.66	-
35	26.7	86.1	106.0	36.0	66	0.60	0.35	1.72	177.00	0.12	5.00	-
36	16.6	50.2	46.0	16.0	65	0.76	0.84	0.91	220.00	0.07	10.85	-
37	14.9	49.3	55.0	20.0	64	0.84	0.67	1.27	161.00	0.09	9.33	-
38	15.6	39.0	64.0	27.0	58	0.78	0.57	1.35	170.00	0.10	8.12	-
39	18.5	72.5	74.0	32.0	56	0.97	0.51	1.91	183.00	0.14	7.10	-
40	21.2	59.7	78.0	29.0	63	0.94	0.81	1.18	168.00	0.10	9.12	-
41	19.0	58.0	62.0	20.0	68	0.83	0.73	1.14	199.00	0.08	10.23	-
42	17.7	78.4	76.0	30.0	61	0.77	0.52	1.50	184.00	0.07	11.84	-
43	27.8	78.4	78.0	29.0	63	0.76	0.84	0.90	220.00	0.08	9.50	-

Raw data spreadsheets

S.No.	Peak aortic velocity (m/sec)	Mean gradient (mmHg)	AV VTI (cm)	LVOT VTI (cm)	AVA (cm^2)	AVAi (cm^2/m^2)	GLS	GCS	GAS	GRS	Basal LS	Apical LS	NT-ProBNP (pg/ml)
1	4.2	45.0	104.2	31.2	0.7	0.5	-14.1	-15.0	-26.0	38.0	-9.0	-20.4	1355.00
2	4.8	58.0	106.0	18.6	0.8	0.5	-17.8	-21.0	-32.0	56.0	-5.2	-27.8	1118.60
3	4.4	44.6	107.0	27.8	0.9	0.5	-10.0	-19.0	-27.0	40.0	-8.8	-13.2	535.20
4	4.7	54.2	113.0	19.0	0.7	0.4	-8.1	-11.0	-19.0	20.0	-6.7	-12.6	2179.00
5	3.2	28.0	79.7	34.3	1.2	0.9	-19.2	-22.0	-35.0	59.0	-15.8	-24.6	117.40
6	3.9	36.9	84.0	20.3	0.7	0.4	-13.0	-14.0	-22.0	32.0	-10.2	-15.6	264.20
7	3.5	28.0	76.0	31.4	1.3	0.7	-19.0	-22.0	-34.0	56.0	-15.2	-26.6	187.70
8	4.7	57.3	100.1	16.4	0.7	0.4	-10.7	-11.0	-18.0	24.0	-6.2	-15.2	331.60
9	4.4	46.9	71.5	19.4	1.0	0.5	-12.2	-16.0	-26.0	38.0	-8.8	-8.6	182.00
10	4.4	44.0	76.7	29.9	0.9	0.5	-11.8	-19.0	-27.0	40.0	-5.2	-13.2	647.70
11	3.4	27.3	76.5	27.5	1.2	0.7	-21.0	-18.0	-34.0	59.0	-20.0	-18.2	54.40
12	3.7	30.6	72.9	25.6	1.3	0.9	-20.5	-20.0	-35.0	57.0	-17.2	-24.4	131.50
13	5.5	77.6	137.0	26.0	0.7	0.4	-7.0	-15.0	-21.0	29.0	-1.5	-7.2	5603.00
14	3.5	31.1	70.7	19.1	1.2	0.6	-9.3	-16.0	-25.0	35.0	-7.7	-7.0	6280.00
15	4.2	40.2	91.4	23.8	0.8	0.4	-12.6	-19.0	-28.0	46.0	-8.5	-12.8	81.65
16	5.9	94.7	155.4	23.8	0.3	0.2	-11.5	-15.0	-20.0	38.0	-12.3	-9.0	4764.00
17	4.6	58.3	98.5	19.5	0.5	0.3	-14.4	-15.0	-27.0	40.0	-8.2	-21.0	151.50
18	3.8	36.3	81.7	20.5	0.6	0.4	-13.0	-16.0	-24.0	36.0	-7.2	-16.4	1381.00
19	4.1	40.5	82.1	19.9	0.8	0.5	-18.0	-23.0	-34.0	60.0	-14.2	-23.2	96.94
20	4.6	51.1	103.8	25.0	0.7	0.4	-10.5	-12.0	-23.0	32.0	-5.7	-16.2	5361.00
21	5.9	88.7	155.2	30.2	0.6	0.5	-12.6	-17.0	-25.0	41.0	-8.3	-12.8	4357.00
22	4.8	56.6	95.2	17.3	0.9	0.5	-8.1	-11.0	-19.0	20.0	-2.2	-12.2	2141.00
23	4.8	54.6	104.1	29.1	0.8	0.5	-15.0	-15.0	-25.0	38.0	-13.3	-16.4	163.40
24	4.6	52.4	91.8	21.5	0.8	0.5	-11.6	-17.0	-26.0	40.0	-3.8	-18.8	453.40
25	4.7	53.7	105.9	28.0	0.8	0.5	-14.5	-18.0	-28.0	43.0	-9.8	-19.8	1191.00
26	5.6	89.1	142.7	14.6	0.8	0.5	-15.2	-18.0	-31.0	50.0	-10.5	-22.4	614.20
27	3.3	25.3	67.5	26.3	1.3	0.7	-14.9	-16.0	-27.0	40.0	-9.2	-17.4	29.05
28	5.2	67.9	133.3	19.0	0.6	0.3	-9.1	-14.0	-21.0	29.0	-6.7	-12.6	3332.00
29	3.6	31.5	77.6	28.3	1.0	0.6	-20.5	-18.0	-31.0	48.0	-16.2	-24.6	120.40
30	4.7	64.2	123.0	24.9	0.7	0.4	-9.9	-19.0	-27.0	40.0	-8.1	-13.6	194.20
31	3.4	29.8	72.6	18.6	0.8	0.5	-13.5	-12.0	-20.0	27.0	-8.7	-18.8	846.00
32	4.6	50.9	86.6	24.9	0.8	0.5	-13.5	-11.0	-20.0	28.0	-12.2	-16.2	70.66
33	4.3	44.5	92.3	23.3	0.9	0.5	-15.2	-16.0	-23.0	34.0	-11.3	-16.2	195.20
34	1.1	2.7	26.6	25.0	3.9	2.0	-19.8	-16.0	-28.0	41.0	-18.2	-22.0	13.30
35	1.0	2.4	28.1	23.6	3.8	2.4	-22.8	-15.0	-32.0	50.0	-17.3	-29.2	22.33
36	1.3	3.8	27.5	28.2	2.9	1.7	-23.4	-20.0	-35.0	59.0	-17.8	-31.8	79.85
37	1.2	2.6	24.4	24.0	2.5	1.6	-18.7	-13.0	-26.0	40.0	-20.0	-18.6	23.04
38	0.9	2.2	23.2	22.2	3.0	1.7	-20.9	-21.0	-34.0	58.0	-19.5	-23.0	13.32
39	1.2	2.3	21.1	20.8	3.1	1.7	-19.5	-16.0	-31.0	49.0	-18.2	-22.2	69.61
40	1.0	2.4	18.6	17.1	3.5	1.7	-20.6	-19.0	-26.0	40.0	-20.2	-21.8	30.03
41	1.1	2.4	21.1	21.2	2.6	1.6	-20.0	-16.0	-34.0	57.0	-18.2	-22.2	39.24
42	1.0	2.3	21.0	17.7	2.3	1.4	-20.2	-13.0	-26.0	38.0	-20.8	-21.8	39.38
43	1.3	3.2	27.2	26.5	3.7	2.3	-21.8	-19.0	-32.0	57.0	-20.0	-21.2	80.82